HEREDITAS

GENETISKT ARKIV

UTGIVET AV MENDELSKA SÄLLSKAPET I LUND

REDAKTÖR: ROBERT LARSSON

BAND XL 1954

LUND 1954, BERLINGSKA BOKTRYCKERIET

HEREDITAS

7P
317

HEREDITAS

GENETISKT ARKIV

UNIVERSITY OF MICHIGAN
APR - 4 1954
SCIENCE LIBRARY

UTGIVET AV MENDELSKA SÄLLSKAPET I LUND

REDAKTÖR: ROBERT LARSSON

BAND XL HÄFT. 1—2

Jan.

LUND 1954, BERLINGSKA BOKTRYCKERIET

HEREDITAS,

a periodical devoted to the publication of original research in genetics and cytology, is published by the MENDELIAN SOCIETY of Lund, Sweden. All contributions are written in English, German or French, illustrated, when necessary, with adequate text figures and plates. The current volume (XL) will contain about 500 pages, issued in four numbers.

Subscriptions should be sent to the Editor. Price *Thirty-five* (*35*) *Swedish Kronor per volume.* Postage free.

Back volumes. — The price of Vols. I—XII, XVIII—XX, and XXIII is 15 Sw. Kr. each, of Vols. XXVI—XXXIII and XXXVII, 25 Sw. Kr. each, and of Vol. XXXIX, 35 Sw. Kr. Postage free. (Vols. XIII, XIV, XV, XVI, XVII, XXI, XXII, XXIV, XXV, XXXIV, XXXV, XXXVI, XXXVIII, and Suppl. Vol. 1949 are entirely out of print.)

ROBERT LARSSON
Editor of »Hereditas»
3 Adelgatan, LUND, SWEDEN.

REDAKTIONSKOMMITTÉ

PROFESSOR, FIL. DR. *ARNE MÜNTZING*
PROFESSOR, FIL. DR. *HERIBERT NILSSON*
PROFESSOR, FIL. DR. *GERT BONNIER*
PROFESSOR, MED. DR. *ERIK ESSEN-MÖLLER*

COLCHICINE-INDUCED C-MITOSIS IN TWO MOUSE ASCITES TUMOURS

BY *ALBERT LEVAN*

CANCER CHROMOSOME LABORATORY, INSTITUTE OF GENETICS, LUND, SWEDEN

I. INTRODUCTION. GENERAL PLANNING OF THE WORK.

ALTHOUGH many experiments on the influence of colchicine on tumour tissue have been reported, so far no coherent analysis of the c-mitotic course has been given for such material, as little as for any mammalian material. Our knowledge of the detailed course of c-mitosis is almost entirely limited to plant material, which is undoubtedly by far more easily approachable for such studies. Such a vital point as whether c-mitosis leads to permanent chromosome doubling, which as far as the plants are concerned constitutes a very commonplace question, remains still a matter of controversy in zoological material. Thus it has sometimes been pleaded that failure to induce chromosome doubling in animal tissue indicates a fundamental difference in the c-mitotic course between animals and plants. While the plant cell immediately after the cease of the c-mitotic effect can go back to normal mitotic activity, the animal cell should be unable to do so, due to disturbances of the centrosomic apparatus. The origin of multipolar spindles should confer chromosomal anarchy to the animal tissue as a direct consequence of the colchicine treatment. The successful cases of colchicine-induced increase in chromosome number in mammals reported by HÄGGQVIST and BANE (1950, 1951) have even been thought to owe their success to the fact that the stage influenced by colchicine was the second meiotic division of the egg, which has a unique situation in regard to the centrosomes, since the subsequent mitosis will receive its centrosomic apparatus from the sperm.

It has been shown (LETTRÉ, *e.g.* 1946; LEVAN and HAUSCHKA, 1952) that certain mouse ascites tumours are characterized by unusually clear chromosome conditions. They are in this respect strikingly superior not only to solid tumours but to every mammalian tissue, with the possible exception of young blastocysts. Therefore ascites cells seem to be a suitable material for analysing c-mitosis in animal tissue, even if it

must be remembered that results obtained in neoplastic tissue cannot be directly generalized to all animal cells. On the other hand, any differences in chromosome reactions between cancer tissue and normal tissue, indicating that the cellular reaction norm has been altered in connection with the incidence of malignancy, would be extremely interesting. A detailed knowledge of the chromosomal and cellular responses to colchicine in representative ascites tumours would be valuable as a first approach to the problem of specific properties of c-mitosis in animals and possibly to a comparison between c-mitosis in malignant and normal tissue.

That ascites cells respond towards colchicine with highly characteristic reactions is well known from various German studies (SCHAIRER, 1940; DITTMAR and MAAS, 1944; the works of the LETTRÉ-school, *e. g.* LETTRÉ, KRAPP and OCHSENSCHLÄGER, 1950). In this last-mentioned paper very clear photomicrographs are given of c-mitosis induced by a colchicine-derivative (N-methyl-colchic-amide). The beautiful analysis of BAYREUTHER (1952) of the idiogram of the German hyper-diploid Ehrlich was performed with exploitation of the mitotic inhibition induced by the same chemical, a feature, which constitutes a typical phase of c-mitosis.

The cytogenetic aspects of c-mitosis have still been only little considered. This involves such questions as whether colchicine treatments may induce permanent changes in the cancer cells and consequently in the composition of the cancer cell population. The studies of KLEIN (1951) and KLEIN, KLEIN and KLEIN (1952) indicate changes of only modificative nature. Even daily treatments with colchicine continued during twelve transplant generations »failed to produce any morphological or chemical change that would have been permanent after the treatment had been abandoned» (KLEIN, 1951, p. 30). The present work was planned with the discrepancy in mind between the chromosome morphology observations, which indicate that colchicine has the ability to induce a possibly specific type of c-mitosis, and the experimental data, indicating that no chromosome doubling is caused by the treatments.

The material for the present study consisted of two ascites tumours both obtained from Dr. GEORG KLEIN's laboratory, Karolinska Institute, Stockholm, and subsequently grown in white hybrid mice at our laboratory. One is originally a retothel-sarcoma, now anaplastic, appearing spontaneously in 1952 in the laboratory of Dr. CHRISTOPH LANDSCHÜTZ, Munich. This tumour, Landschütz I, is a hyperdiploid,

which both in chromosome number and in chromosome morphology shows great similarities to the Ehrlich-type studied by BAYREUTHER (*l. c.*). Its chromosome conditions are analysed by TJIO and LEVAN (1954). The other tumour used in the present study is the tetraploid Ehrlich, studied by LEVAN and HAUSCHKA (1952). Although it might have been of interest to choose a purely diploid tumour together with the tetraploid one, I decided to use Landschütz I mainly because of its extremely favourable cytologic conditions.

The colchicine treatments were made in the following way. A certain quantity, usually 0,17 cc per 10 g body weight of Ringer solution, in which the colchicine was dissolved, was injected intraperitoneally into animals, inoculated 6—9 days earlier with the tumour. Samples of the ascites fluid were drawn after different time intervals and studied as aceto-orcein squashes. It might have been desirable to make the treatments in tumours of equal age, as, for instance, always on the sixth day after inoculation. This turned out to be impracticable, however, especially in cases where many samples had to be taken in a short time of the same animal. It was necessary to wait with the treatment, until a sufficient quantity of ascites fluid had been formed. Usually this took somewhat longer in Landschütz than in Ehrlich.

The concentrations of colchicine were constantly calculated as amount of colchicine injected per body weight. In this paper the concentrations will always be given as molar concentrations times 10^{-9} per kg body weight. These values are converted into γ/10 g by multiplying with 0,004. For facilitating the conversion of the values given here into γ/10 g and parts per million the following table is submitted:

10^{-9} mol/1000 g	γ/10 g	One part in the following number of million parts
1,25	0,005	2000
12,5	0,05	200
25	0,1	100
40	0,16	60
50	0 2	48
100	0,4	24
125	0,5	20
200	0,8	12
400	1,6	6
1250	5	2
2500	10	1
3750	15	0,67
12500	50	0,2

The following six experiments were performed:

Experiment 1 (started on June 23). One male inoculated with Ehrlich was treated with concentration 3750. Samples for fixation were taken at the following times after the injection of the colchicine dose: 0 (control), 10, 20, 30, 45 minutes, 1, 1,5, 3, 6, 20 and 26 hours. A general orientation of the c-mitotic effects was expected from this experiment.

Experiment 2 (started on June 29). Five females inoculated with Ehrlich were treated with the concentrations 1,25, 12,5, 125, 1250 and 12500. Fixations were taken 0, 10, 20, 40 minutes, $1^1/_3$, $2^2/_3$, $5^1/_3$, $10^2/_3$ hours, 1, $2^1/_6$, 3, 4 and 5 days after the treatment. The experiment was planned for estimating the threshold concentration region of c-mitosis.

Experiment 3 (started on July 11). *a. Single treatment.* Five males inoculated with Ehrlich were treated with concentrations 25, 50, 100, 200 and 400. Fixations were made at 0, 1, 2, 4, 8 hours, $1^2/_6$, $2^1/_{24}$ and 3 days. The experiment aimed at a closer study of the c-mitotic threshold zone. — *b. Repeated treatment.* Four males inoculated with Ehrlich were given daily injections with the concentrations 50, 100, 200 and 400 during 3 days. Fixations were made the last day of treatment and on one day later. Recovery phenomena of c-mitosis were sought.

Experiment 4 (started on July 4). Four animals inoculated with Landschütz I were treated with the concentrations 40, 125, 400 and 1250. Fixations were made at 0, 2, 5 hours, $1^1/_{24}$, $1^3/_4$, $2^1/_{24}$, $2^5/_6$, $5^1/_{24}$ days, for a comparison between Landschütz and Ehrlich.

Experiment 5 (started on August 3). Two mice inoculated with Ehrlich were treated with the concentrations 1250 and 2500. Fixations were made after 0, $7^7/_{12}$ hours, 1, 2, 3 and $4^1/_8$ days, an experiment for a closer study of the recovery phenomena.

Experiment 6 (started on August 10). *a. Repeated treatment.* Two animals inoculated with Landschütz I and two with Ehrlich were treated daily from the third to the eleventh day after inoculation with concentrations 1250 and 2500, respectively. From day 6 after inoculation daily attempts were made for sampling the ascites. Ascites tumour was developing only in one of the Ehrlich-inoculated animals. — *b. Single treatment.* The same number of inoculated animals were subjected to one single treatment with the same concentrations as in *a.* Fixations were made after 0, 6, 12, 24, 36 hours, 2, 3, 4 and 6 days. The experiment was directed towards possible differences in the recovery process between Landschütz and Ehrlich. — *c. Transplantation experiment with colchicine-treated inoculate.* Four animals were inoculated with Landschütz I ascites derived from experiment 6 *b* on its 6th day. These

animals gave tumours which could be sampled from September 2. The experiment was undertaken for prolonging the period of observation of the recovery phenomena over the life span of the treated animals.

The present investigation was made possible by a grant from the Swedish Anti-Cancer Society. My thanks are also due to Professor T. CASPERSSON and Dr. GEORG KLEIN for constant help and advice and for material, to Dr. GUNNAR ÖSTERGREN for constructive criticism, and to my colleagues at the Cancer Chromosome Laboratory, Mrs. EVA MELANDER and Dr. JOE HIN TJIO, as well as to Dr. W. S. PLAUT for valuable help.

II. DETERMINATION OF THE THRESHOLD CONCENTRATION OF C-MITOSIS.

1. EARLIER EXPERIENCES.

It is possible to obtain from the literature a fair idea of the concentration zone in which the threshold for c-mitosis induced by colchicine should be expected. Without aiming at completeness some instances of earlier work on colchicine treatments of mouse tumour tissue will be mentioned.

LUDFORD (1936) treated various mouse carcinomas in tissue culture with colchicine in concentrations between 2,5 and 25000 (given as molar concentrations $\times 10^{-9}$ per kg). Mitotic inhibition was observed in concentrations above 62,5. In the nearest lower concentration, 25, mitotic inhibition was seen in some cells, normal mitosis in others. The mitotic inhibition led to an increase in the number of mitoses present. Clear c-mitoses of star-type were pictured (Fig. 5, p. 434) from treatments with 312,5. Above 625 toxic action started to appear, above 3125 this became more pronounced: mitoses were rare, outgrowth from the explantate was inhibited. The toxic effect was very strong in 25000, in which concentration the culture degenerated in 24 hours.

Working with mouse sarcoma 180, BRUES and JACKSON (1937) found that a dose of 3125 results in a pronounced accumulation of mitoses (mitotic index went up to 12 instead of the usual 1—2).

In his experiments with the Ehrlich carcinoma, SCHAIRER (1940) gave two doses on the third and seventh day after inoculation. Each dose was 3750 or 7500. Usually the animals died a few hours after the second dose. The mitoses of the ascites cells in animals thus treated exhibit very striking effects: »Am häufigsten sah man, dass die Kern-

membran völlig aufgelöst war und dass nun die Chromosomen völlig isoliert von einander oder auch in kleinen zusammenhängenden Gruppen schachbrettartig in den Zellen verteilt waren» (*l. c.*, p. 146). Multinucleate cells with as many as 20—30 nuclei were frequent.

TENNANT and LIEBOW (1940) treated tissue cultures of a mouse carcinoma (Yale carcinoma No. 1) with colchicine concentrations from 4,9 to 1250. 312,5 effected already after 30 minutes very strong cytological changes: »All the mitotic figures were found to be comprised of short thick chromosomes arranged in a roughly spherical mass near the center of the cell ... Within an hour the chromosomes in some cells became much shorter and were widely dispersed throughout the cytoplasm, as described by LUDFORD» (*l. c.*, p. 41). This latter chromosome arrangement is evidently the same as SCHAIRER describes as »schachbrettartig». Similar changes were induced by concentrations down to 19,5, but in these low concentrations they appeared only after a somewhat longer time (16 hours). The growth of the colonies was noticeably reduced by concentrations above 38.

LETTRÉ, KRAPP and OCHSENSCHLÄGER (1950) report an increase in the mitotic index within the Ehrlich tumour with a colchicine concentration above 625. This increase culminates at 6250. It is of interest that this maximum of mitotic blocking occurs earlier (after 10—13 hours' treatment) in lower concentrations (3750 and lower) and later (after 24—36 hours) in higher concentrations. The authors give the toxic dose as 6250 (*i. e.* the highest non-lethal dose). Even at very strong doses, as 100000, death is not instantaneous but occurs after about 20 hours.

KLEIN (1951) and KLEIN, KLEIN and KLEIN (1952) injected daily from the third day after the inoculation with Ehrlich a dose of 1500 and, from the seventh day, 2000. Ascites developed 12 to 16 days after inoculation, the animals were killed the day after the last injection. This means in some cases a total dose of 25000 for each animal. The ascites contained a high frequency of multinucleate cells (45,4 % as against 0,9 % in untreated animals).

Recently BACK and WALASZEK (1953) treated the Ehrlich ascites with radioactive colchicine. They injected immense single doses of the size order of 62500 and 187500. The animals were killed after 4 hours.

The experiences of various workers now mentioned are concordant enough to allow a certain idea as to the concentration zone, in which the c-mitotic threshold may be expected. Clear c-mitotic effects were reported from 25—62,5 by LUDFORD, from 19,5—312,5 by TENNANT and

LIEBOW. Very pronounced c-mitosis can be inferred from all experiments with stronger concentrations, as in 3125 (BRUES and JACKSON), 3750 (SCHAIRER), 625—6250 (LETTRÉ, KRAPP and OCHSENSCHLÄGER). Evidently the concentration zone 10—5000 is the one which should be first looked into.

2. THE PRESENT EXPERIMENTS.

The colchicine effect on the ascites cells was estimated in the following way. A number of cells from each sample was investigated under oil immersion. The normal cell number analysed each time was 1000, in Experiment 1 it was 2500, in exceptional cases, where the scarcity of cells made observations difficult, only 500 cells. The cells were counted as resting stages or mitotic stages. The latter were divided into the different mitotic stages, and in addition into three groups: (1) normal mitoses, (2) colchicine-influenced mitoses, and (3) regular c-mitoses. Only those were counted as normal in which typical mitotic stages evidently were under full spindle control. As soon as any spindle disturbances were met with, the cells were included in group (2), although such disturbances are often found spontaneously in cancer cells. As group (3) were counted typical c-mitoses as well as those deviations of c-mitosis, in which the spindle has some influence, as star-, ball-, exploded, *etc.* mitoses. Group (3) was in addition subdivided into ordinary c-mitoses and scattered c-mitoses, the latter ones being extremely characteristic of ascites cells. On calculating the c-mitotic percentage the groups (2) and (3) were added and fractioned on the total number of mitoses recorded. Prophases were excluded from this calculation, however, since it is almost impossible to distinguish with certainty between a c-prophase and a normal prophase.

This method of counting makes the c-mitotic percentage a maximal value, it includes both spontaneous spindle disturbances and normal pro-metaphases, which, as is well recognized, can hardly be distinguished from certain c-mitotic stages. Thus BUCHER (1945 a) in estimating the action of colchicine on rabbit fibrocytes grown *in vitro* reports a striking increase between the concentrations 41,5 and 62,5 of early metaphases »mit noch ungeordneten Chromosomen». It may safely be assumed that this group also includes c-metaphases and that the concentration zone mentioned is the c-mitotic threshold zone in this case. The only safe criterion for c-mitosis is the failing anaphase movement, as the normal bipolar anaphase is the only criterion for the absence of c-mitosis. A certain measure of control on the usefulness of the classific-

ation method here employed was gained from the counts made at the start of every experiment. These control counts, 20 in number, gave normally 0 as c-mitotic percentage, in four cases c-mitotic percentages varying from 3,8 to 12,5 were found, which consequently may be expected to occur spontaneously in the tumour material.

In all, the c-mitotic percentage was counted in 165 samples (123 in Ehrlich, 42 in Landschütz), comprising altogether 173500 cells (135000 in Ehrlich, 38500 in Landschütz). It was evident from the material recorded that this type of treatment gives fairly reproducible results; the same colchicine dose injected on different occasions into different animals will give reasonably concordant c-mitotic percentages. The sources of error are of course considerable. Thus it is clear that here, even more than in the *Allium* test, the colchicine concentration surrounding the individual cell is unknown.

The c-mitotic percentages recorded are collected in Table 1. In certain cases the values of this table represent averages of several experimental series (Ehrlich 125, 3 series; 1250, 3 series; 2500, 2 series; Landschütz 1250, 2 series), and also to a certain degree averages of times of treatment. From the table is seen that the induction of c-mitosis depends on two factors: the concentration and the time of treatment. The lowest concentration recorded, 12,5, has a c-mitotic incidence hardly distinguishable from that of the controls. Already at 25, however, the c-mitotic percentage is without any doubt caused by the treatments. At 400 total c-mitosis is induced by one hour's treatment. With increasing concentration the time interval required for obtaining total c-mitosis is shortened, at the highest concentration studied, 12500, this interval is 20 minutes.

It is seen from the table that the initial stages of the treatments induce fairly regular c-mitotic effects. In Fig. 1 a graphic representation of the increase with time of the c-mitotic percentages is given for five colchicine concentrations. Considering the variations expected from the uncertainty of knowing the real concentration in the cancer cells and other such sources of error, a fairly good proportionality is shown between dosage and effect. This is especially apparent if the values of different times of treatment are correlated with the concentrations employed. Each time of treatment results in an S-curve (Fig. 2). It is evident that the curves for the longer times of treatment are pulled downwards, indicating the gradual elimination of the drug from the body.

In the highest concentrations c-mitosis reaches 100 % and remains

TABLE 1. *C-mitosis percentage at certain time intervals after the injection of various colchicine dosages.*

Time after treatment	Ehrlich											Landschütz				
	Concentrations											Concentrations				
	12,5	25	50	100	125	200	400	1250	2500	3750	12500	40	125	400	1250	2500
30 min.	7,7	—	—	—	23,6	—	—	74,7	—	57,2	88,5	—	—	—	—	—
1 h	3,2	28,6	—	20,0	76,9	62,5	100	88,9	—	96,9	100	—	—	—	—	—
2 h	0	35,7	28,0	53,8	94,6	74,1	100	100	—	100	90,9	26,7	42,3	100	100	—
3 h	0	—	—	—	93,3	—	—	100	—	100	100	—	—	—	—	—
6 h	3,7	31,7	39,3	75,8	95,1	78,6	98,2	100	100	100	100	55,6	93,3	100	100	100
12 h	0	29,6	41,7	87,1	74,3	58,5	94,9	95,9	100	—	—	—	—	—	100	100
24 h	11,5	41,2	33,3	40,0	67,8	44.1	75,0	54,7	87,3	100	100	—	—	—	88,4	97,1
36 h	—	50,0	53,8	81,3	—	50,0	89,4	44,0	50,0	100	—	46,9	55,6	75,0	84,7	75,0
2 d	—	—	—	—	—	—	—	47,9	72,3	—	100	44,8	50,0	35,6	83,4	59,0
3 d	—	—	96,1	92,2	58,2	44,8	49,9	20,7	46,9	—	—	17,9	—	20,0	58,7	52,0
4 d	—	—	—	—	32,5	—	—	16,0	25,8	—	—	—	—	—	35,0	35,0
6 d	—	—	—	—	19,2	—	—	23,5	70,0	—	—	—	—	—	63,9	—

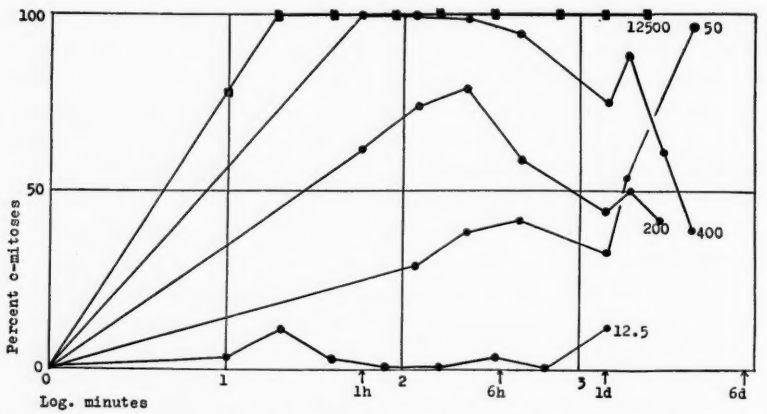


Fig. 1. C-mitosis percentage at different time intervals after the injection of colchicine doses varying from 12,5 to 12500$\times 10^{-9}$ mol/kg.

at that value during the experiment. Between concentrations 400 and 2500 100 % c-mitosis was attained during the first 12 hours of treatment, but after that the percentage again went below 100. Curves of the type found in concentration 400 in Fig. 1 are obtained. This tendency of the curves is considered to be the typical case. It is striking, however, how the part of the curves corresponding to the recovery of the c-mitotic

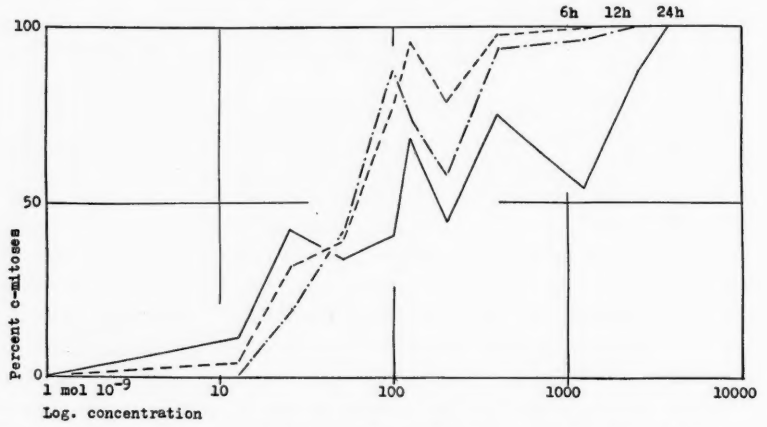


Fig. 2. C-mitosis percentage of different doses after the lapse of 6, 12 and 24 hours, respectively.

effects is decidedly less regular than the curves for the initial period. Quite unexpected irregularities are met with; the curves for the concentrations 50 and 100, for instance, show a very clear increasing tendency towards the end of the experiments. Considering the profound changes certain fractions of the cellular population may undergo under the influence of the polyploidogenic, and perhaps mutagenic, action of the colchicine, it is hardly unexpected that this in some cases will express itself as irregularities of the spindle function, which on recording will appear as an increased c-mitotic percentage. Evidently we have to reckon with partly a primary induction of c-mitosis, which obeys a certain regularity, and partly secondary types of c-mitosis, which are more unaccountable in their appearance, and which do not follow any strict dosage-correlation.

My determinations of the c-mitotic percentage allow an estimation of the c-mitotic threshold for the present tumours. The concentration 1,25 gave no appreciable c-mitosis, 12,5 gave weak tendencies, not with certainty differing from the untreated material. From 25 upwards c-mitosis is induced by the treatments. If by the threshold value is meant the zone between the highest concentration without an effect and the lowest concentration with an undisputable effect, this zone for Ehrlich will be 12,5—25. Landschütz was not studied in such low concentrations. If the zone between the highest concentration with only incomplete c-mitosis and the lowest concentration giving total c-mitosis is considered as threshold zone, then 200—400 will be the threshold in our case. Owing to the uncertainty in the present kind of experiments it may be safe only to conclude that the threshold concentration is of the size order of 10^{-7} mol/1000 g.

In Fig. 3 the maximal values attained by the c-mitotic percentage in different concentrations have been plotted (excluding the late high values in 50 and 100 discussed above). This gives a good picture of the threshold region as it has been conceived in my experiments. In the same graph there has also been included the threshold values for c-mitosis in root meristem cells of *Allium Cepa*, although, of course, the concentrations in the two cases are not directly comparable. The threshold zone of the ascites cells is lower than in *Allium*, and in addition much more extended. On passing from a c-mitotic percentage of 0 to 100 the following concentrations have to be passed in the two materials (all expressed as 10^{-9} mol/1000 g):

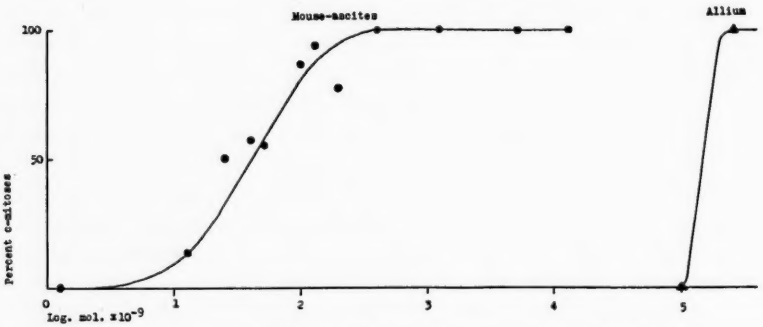


Fig. 3. The c-mitotic threshold regions of mouse ascites and of *Allium* root meristems.

c-mitotic %	0	100
Ascites	1,25	400
Allium	100000	250000

The relation of c-mitotic percentages 100 : 0 is for ascites 320, for *Allium* 2,5. If 25 is counted as threshold value for the ascites and 125000 for *Allium* the latter material is 5000 times less sensitive to the c-mitotic action of colchicine. This relation will be still much greater for the toxic thresholds of colchicine.

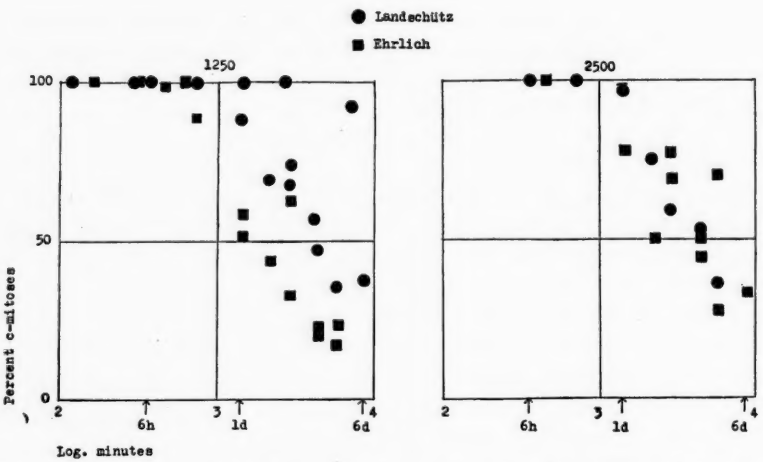


Fig. 4. C-mitosis percentages during the recovery period after treatment with the doses 1250 (left graph) and 2500 (right graph).

In Table 1 accessible values are included from both Ehrlich and Landschütz allowing a comparison of the two. No striking differences are seen in their c-mitotic threshold values. Total c-mitosis is attained at the same minimum dose. During the recovery, however, certain indications are found that Landschütz may be somewhat slower in returning to normal mitosis than Ehrlich (*i. e.* Landschütz may be somewhat more sensitive to colchicine). Several comparable values for both tumours exist for the two concentrations 1250 and 2500. They are collected on the graph of Fig. 4. At least in the concentration 1250 this material indicates a difference between the two tumours: all c-mitotic percentages of Landschütz are situated above the corresponding values of Ehrlich. At the concentration 2500 the situation is more uncertain.

III. THE MORPHOLOGY OF THE C-MITOTIC REACTION IN THE ASCITES CELLS.

Characteristic modifications of the mitoses going on at the start of the treatment were encountered already at the first samplings in treatments above the threshold concentration, *i. e.* after ten minutes. The chromosomes were losing their regular arrangement on the spindle, in strong concentrations this happened in every metaphase or early anaphase. The penetration of the colchicine evidently takes place rapidly.

The effect of the colchicine on those cells already in division at the start of the treatment is typical: as the pulling of the spindle is relaxed, the chromosomes gather into a cluster in the centre of the cell. This immediate reaction is pictured in Fig. 5 *a—f*. Often several chromosomes are collected with their centromeres meeting in one point and their bodies radially arranged; in extreme cases all chromosomes of one cell are thus arranged, as the star-metaphase of Fig. 5 *d*. Ball-metaphases with all chromosomes packed in the centre of the cell are frequent (Fig. 5 *f*). If the c-effect is combined with a certain stickiness, the metaphase chromosomes may keep their old arrangement on the equatorial plate after the spindle has ceased to function. Such configurations, more or less pycnotic metaphase plates, may often be seen in untreated material and are especially frequent in certain treatments (Fig. 5 *e*).

This early type of c-mitosis is characteristic of short treatments, it is called here *initial c-mitosis*. Already after one hour the c-mitoses begin to shift character, however. Instead of gathering in the centre of the cell the c-pairs are scattered out all over the plasm. This is the

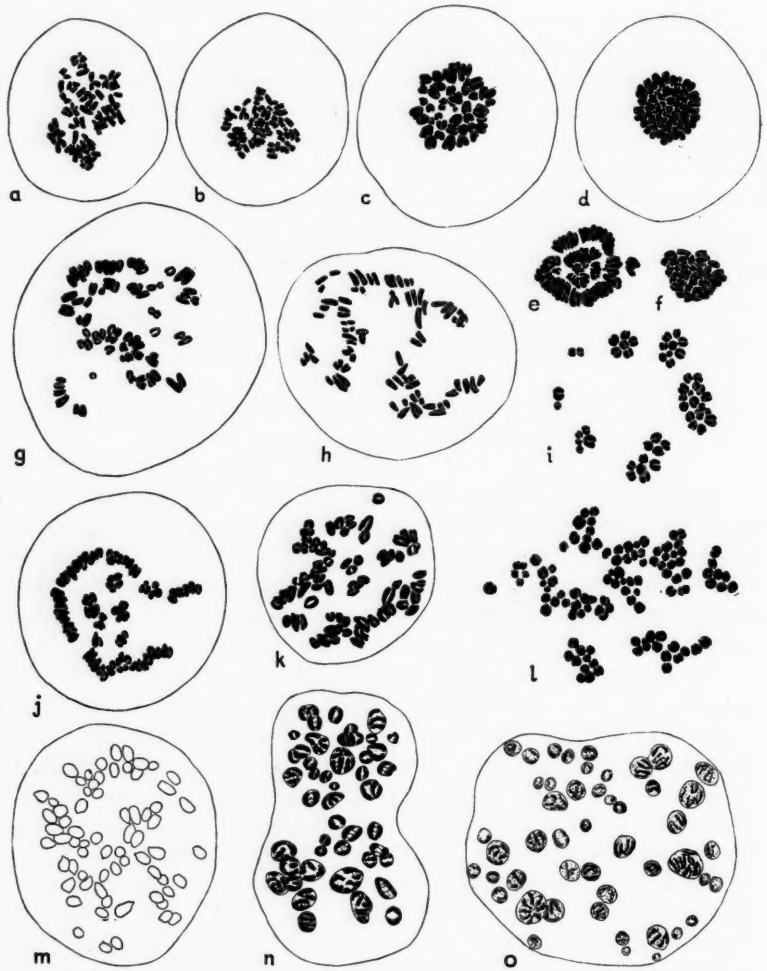


Fig. 5. *a—f*: the initial c-mitosis; *d*: star metaphase, *e*: coin roll type, *f*: ball metaphase; *g—l*: transitional types between initial and scattered c-mitosis, *i*: several groups of c-pairs in star arrangement, *j*: coin roll type; *m*: scattered c-metaphase; *n—o*: scattered c-telophase. *Treatments*: *a*, *b*, *d—f*, *h*, *k*, *m—n*: 3750, $^1/_2$—6 hours, *c*: 1250, 2 hours, *g*, *j*: 12,5, 3 days+125, 2 days, *i*, *l*, *o*: untreated. Material: *c*, *g*, *j*: Landschütz, the rest: Ehrlich. — ×820.

striking type of c-mitosis so often commented upon by various writers as »chessboard arrangement», »scattering of the chromosomes», *etc.* Here this type will be referred to as *scattered c-mitosis.* The condition leading to this type of c-mitosis is that prophase chromosomes are released into a big cell without any spindle action. Probably a contributory factor in this situation is that the prophase chromosomes under the influence of the colchicine already within the nuclear membrane are shortened and therefore are less likely to touch each other when the membrane disappears. Fig. 5 *g—h* picture transitional forms between the initial and the scattered c-mitosis. In both a weak spindle effect may be traced, but the tendency to scatter out over the cell is evident. Pronounced cases of scattered c-mitosis are shown in Fig. 5 *m* and Fig. 6 *a*, *c* and *e* and in the photomicrographs of Fig. 11 *a—c*.

It should be noted that in the scattered types of c-mitosis a tendency to grouping or more or less regular orientation of the chromosomes is often seen, sometimes indicating a weak spindle action. This is undoubtedly the case with Fig. 5 *i*, where several groups of radially arranged chromosomes are found, within each group all chromosomes turn their centromeric end inwards towards one point. More occasional groupings are represented by Fig. 5 *k* and *l*, while Fig. 5 *j* constitutes a more scattered case of the same phenomenon as described above for Fig. 5 *e*. Most of the metaphase chromosomes of Fig. 5 *j* are arranged in a wide ring with their bodies parallel and their centromeres turned inwards. This ring is broken in several places leaving the chromosomes in very characteristic groups recalling rolls of coins. This type is common in certain treatments and may appear under many modifications.

At full c-mitosis both of the initial and the scattered type the chromosome contraction is more or less pronounced. Often an extreme contraction leaves the chromosomes completely spherical (Fig. 5 *l*). This type is well-known from untreated tumour cells and may also be found in normal tissue. Such chromosomes, often called ball-chromosomes, arise in connection with spindle disturbances. KOLLER (1947) pictures such cells from human carcinomas (*l. c.*, Figs. 5 and 6). He attributes this »over-condensation» and »over-spiralisation» to an excess of nucleic acid charge on the chromosomes and to failure of spindle formation.

It is interesting to observe that every chromosome modification induced by colchicine can also be found in untreated tumours, only more rarely. The scattered c-mitoses of Fig. 5 *i*, *l* and Fig. 6 *c*, *d* derive from untreated ascites cells. They agree in every detail with the pictures

met with in colchicine-treated material, and there is little doubt that their method of induction is the same in both cases, *viz.* failure of the spindle. It must be pointed out that the morphology of the c-mitosis is highly variable. This is especially true of the chromosome contraction. The cells here pictured are only typical instances.

The typical course of c-mitosis in the present material differs in one striking respect from the c-mitosis, as we know it in plants; it is devoid of c-anaphase, the c-metaphase chromosomes enter telophase directly without division of the centromeres and without falling apart of the chromatids. The »ski-pair» stage, so striking in plants, is lacking. As will be shown below, this stage may be found, but only as exceptions.

In typical cases the initial c-mitoses form more or less pycnotic telophases, usually the whole chromosome cluster forms one restitution nucleus. In this process it is difficult to follow the behaviour of single chromosomes. It is certain, however, that no c-anaphase occurs. In the scattered c-mitoses, on the other hand, it is very easy to follow the transition metaphase—telophase with all its extremely characteristic changes. In some cases each single metaphase chromosome forms a telophase nucleus of its own, in other cases two or more chromosomes group together in forming their telophase nucleus. Both ways lead to the multinucleate telophase stage often referred to in the literature.

Fig. 6 gives a representation of the typical changes from metaphase to telophase in the scattered c-mitosis. Fig. 6 *a*, *c* and *e* show three typical cells with progressive telophase changes. Their chromosomes have been separately drawn at a higher magnification in Fig. 6 *b*, *d* and *f* in order to show the variation in appearance within the same plate. In Fig. 6 *g*—*l* instances of c-pairs at different developmental stages are given. The typical course of telophase changes is the following. Already at metaphase the chromosomes often start to develop a typical form, distinguished from the ordinary metaphase chromosome form. The chromatids touch each other at both ends but fall apart in the middle. This chromosome type has sometimes been referred to as »ring chromosomes». Some tendencies to this may be seen in Fig. 6 *b*. Gradually this central cavity increases, the two chromatids form the lateral walls of it, a nuclear membrane is developed all around, and each metaphase chromosome goes directly over into one telophase nucleus. In those cases where the two metaphase chromatids are surrounded by a common pellicle it appears as if this directly forms the nuclear membrane.

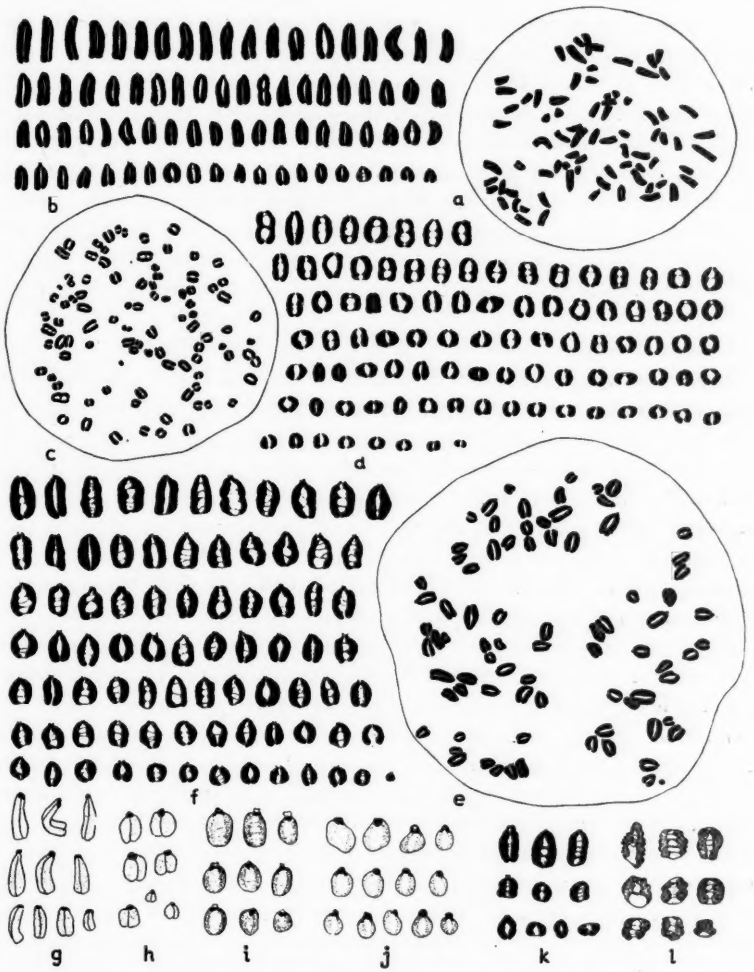


Fig. 6. *a—f*: idiogram analyses of three scattered c-metaphases to telophases; *g—l*: instances of chromosomes from scattered c-metaphases and c-telophases. *Treatments*: *a*, *b*: 3750, 45 minutes, *c—d*: untreated, *e*, *f*, *i—l*: 3750, 6 hours, *g*: 125, 12 hours, *h*: 1250, 2 hours. *Material*: *c*, *d*: Krebs 2, *h*: Landschütz, the rest: Ehrlich. — *a*, *c*, *e*: ×820, the rest ×1600.

During the process of the central falling-apart of the chromatids very characteristic transverse threads run over between them (Fig. 6 *d*, *f*, *k*, Fig. 11 *c*). These threads are evidently chromatic and may indicate especially sticky places on the chromosomes. They remain even after the stainability has become very weak (Fig. 6 *i*, *l*). In certain plates only a few chromosomes show one or two clear transverse connections. They may represent certain heterochromatic sections with strong tendency towards stickiness and they may even become useful as landmarks in the idiogram. A similar behaviour was described by TSCHERMAK (1943) for colchicine-treated *Oedogonium*-chromosomes (*e. g.* Fig. 7 *k*—*m*; *l. c.*, p. 510).

Another characteristic peculiarity of these telophase nuclei is the behaviour of the centromeric region (Fig. 6 *g*—*j*). It not only keeps its stainability much longer than the rest of the chromosome, it evidently also maintains a stronger spiralization even after the telophase nucleus has formed a rounded vesicle. On this vesicle the centromeric region stands out like a protruding papilla, usually rather darkly stained. It may be seen in certain cases that the extreme (=most proximal) part of this papilla is less darkly stained than the basal part of it plus the proximal part of the vesicle. This fits in very well with the picture of the structure of the centromeric region of these chromosomes obtained from other treatments. The most proximal part of the centromere is divided and widely separated already at metaphase. It is not heterochromatic, while the less proximal part of the centromere, still undivided or at least not fallen apart during metaphase, is strongly heterochromatic. It shows »nucleic acid starvation» in certain treatments (TJIO and LEVAN, 1954). The heterochromatic centromeric regions, which are so evident during later stages of the scattered c-mitosis, may be seen also in ordinary telophase nuclei, their detailed structure is more difficult to study here, however, due to the crowding of the chromosomes. Entire cells at telophase are pictured in Fig. 5 *n*, *o*, details of the former one in Fig. 6 *l*. The chromatids are still recognizable, it can be seen that each nucleus contains one or a few entire chromosomes.

Exceptionally there may be found another type of c-mitosis, more in agreement with the ordinary c-mitosis of higher plants. Fig. 7 gives a few pictures of this type. Here the chromatids fall apart early during metaphase (Fig. 7 *a*, *b*), they are then contracted, still widely separated (Fig. 7 *c*, *d*) forming conventional c-pairs, only held together at the terminal centromeres. Eventually in single cases also real c-anaphases are found, in which the centromeres have divided, leaving the chromatids

Fig. 7. The »plant type» of c-mitosis. *a—f*: idiogram analyses of three cells, *g—i*: instances of chromosomes from different metaphase stages, *j*: c-anaphase, *k*: c-pairs from *j*: *Treatment*: 125, 5 1/3 hours. *Material*: Ehrlich. — *a*, *c*, *e*, *j* ×820, the rest × 1600.

free from each other as »ski-pairs». It is not certain that the centromeres are divided in Fig. 7 *e*, *f*, the mutual position of the chromatids suggests that they still hang together, although the connection between them is colourless and extended. The single chromatid at 6 o'clock

(Fig. 7 *e*) must be separated from its partner, found farther in within the plate, but it may have become dislocated at the pressing-out of the slide. Fig. 7 *j*, however, represents a safe c-anaphase, within which the daughter chromatids generally have moved away from each other in such a manner that there can be no doubt that they are entirely free (Fig. 7 *k*).

The last-mentioned type of c-mitosis has been met with in different experimental series. During the whole investigation about 25 certain cases of real c-anaphases were recorded. It seems that it is found in a somewhat higher frequency in weak treatments with dosages close to the threshold value. It was found in the following dosages: concentration 100 for 1—2 days, 125 for 3—24 hours, 200 for 2 days and 400 for 1—2 days. Even here only the metaphase type preceding this anaphase was common, real c-anaphases were rare. Since also normal bipolar anaphases were present in the treatments mentioned, it is often difficult to decide whether an apparent c-anaphase is in reality only the start of a normal anaphase. The chromosomes were in these cases long, extended and hairy. Secondary constrictions were a characteristic feature (Fig. 7 *b*, *h*). Although the chromosomes of this type evidently were somewhat influenced by the spindle, they were usually not arranged into a normal equatorial plate. It is interesting to note that the division of the centromere, which in higher plants takes place in all forms of c-mitosis, here in the ascites cells seems only to be possible under a very weak c-mitotic action.

IV. IMMEDIATE CONSEQUENCES OF THE C-MITOSIS.

1. GENERAL APPROACH.

In those cases where the c-mitosis reaches a high frequency it leads to immediate important and striking consequences to the cancer cell population. Since these changes are caused by various sides of the same c-mitotic process, they are woven into each other and often directly connected with each other. Thus, a modification is induced in the relative frequency of the various mitotic stages, present in the tissue. We have seen that the metaphase stage only exceptionally leads to centromeric division, that is to anaphase. Instead the metaphase, after a longer time interval than usually, goes over into telophase. Therefore the number of metaphases is increased: this is the famous metaphase-blocking, often discussed in the colchicine literature. Simultaneously an increased number of mitoses will accumulate, in other words, the

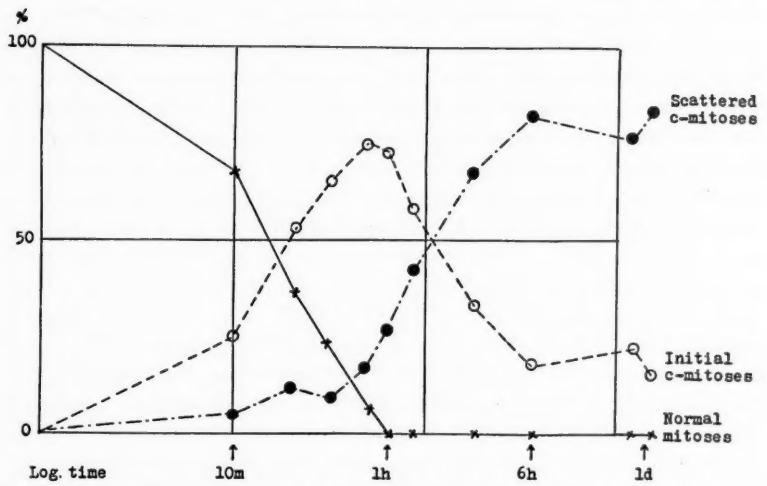


Fig. 8. The frequencies of initial and scattered c-mitosis at certain time intervals after the injection of a colchicine dose of 3750×10^{-9} mol/kg.

mitotic index goes up. This was the most striking effect of colchicine, at first interpreted as a mitotic stimulation, recognized already by DIXON (1905) and further worked out by DUSTIN senior (*e. g.* 1934). The induction of the multinucleate stage, so characteristic of colchicine treatments in ascites, is directly caused by the peculiar type of c-mitosis, analysed in the preceding chapter under the term scattered c-mitosis. This c-mitotic form in its turn is a consequence of the spatial conditions valid at the moment when the c-contracted prophase chromosomes are released into the comparatively large cellular space of the cancer cell. It follows the initial type of c-mitosis in a regular sequence. One instance of the time relationships of these two c-mitotic types (from Experiment 1) is reproduced graphically in Fig. 8.

The present chapter will deal with the consequences of c-mitosis as they have appeared in my material. As basis for this analysis are data from the cells already used for the estimation of the c-mitotic threshold. In these cell counts there was recorded a number of 7165 mitoses, classified as to their mitotic stage. Certain circumstances connected with this classification must be mentioned before entering into the analysis. Thus, a certain difficulty arose at the recording of the prophases. Both Ehrlich and Landschütz contain spontaneously a varying frequency of resting nuclei with endomitotic structures. It is not ex-

cluded that these endomitoses are also influenced by the treatments. An influence on chromosomes at resting stage by the colchicine has already been suggested by RIES (1939), who postulates that colchicine may effect a condensation of the chromocentra, »dass durch Colchicin in Ruhekernen mit weitgehend verteiltem Chromatin die Chromosomen als Individualitäten hervorgerufen werden» (*l. c.*, p. 509). In the present case it sometimes appeared as if endomitotic stages could directly go over into c-metaphases of the scattered type. If that is the case, it might have been more correct to record them as prophases. On the other hand, colchicine treatments bring about a stronger contraction than usually to the ordinary prophasic chromosomes, their appearance will then approach that of the endomitotic chromosomes. Now, the difficulty of distinguishing colchicine-affected prophases from endomitotic contraction stages is mainly present in the strongly modified samples, in which a very high frequency of the cells (33 %) show mitotic activity. I have consistently tried to record as prophases only those belonging to the regular mitotic cycle. The prophase countings are thus minimum values, which in the strongest affected samples may be somewhat too low. The question concerning the reaction of the endomitotic chromosomes toward colchicine is important and should be subjected to a special study, in which some of the ascites tumours with high incidence of endomitosis should be a profitable material.

I hesitated somewhat about the grouping of the data collected. The material is evidently highly heterogeneous: it can hardly be convenient only to divide it into two groups, controls *versus* treatments. This latter group includes concentrations which are so low as to make them more comparable to the controls than to the stronger treatments. Furthermore, the stronger treatments include short times of treatment, during which the effect is progressive, longer treatments where the effect is culminating, and still longer treatments where again normal conditions start to predominate, as the drug is being eliminated by the body. I decided to divide the material into the following five groups:

(1) The controls.

(2) Treatments in which c-mitosis never reaches 100 %. This group includes concentration 200 and lower concentrations.

(3)—(5). Treatments in which c-mitosis reaches 100 %. Concentrations 400—12500. The following three groups are distinguished:

(3) Treatments up to 3 hours.

(4) Treatments from 3 hours to 24 hours.

(5) Treatments longer than 24 hours.

That I decided upon 24 hours as a turning point for the stronger treatments was because of the fact that after this time recovery starts in concentrations up to 2500. It might have been advantageous to sift out the two highest concentrations, 3750 and 12500, as a group of their own. These two concentrations behave in many respects similarly and distinct from the rest of the groups (3)—(5). However, the material would probably have lost more in surveyability by this than would have been gained in other respects. In all groups Ehrlich and Landschütz were treated separately.

2. MITOTIC STIMULATION?

It was possible to illuminate somewhat the question of a mitotic stimulation by colchicine in my material. The number of prophases in 1000 cells constitutes under normal conditions a measure of the intensity with which the tissue enters mitosis. If an increase in number is effected under the colchicine influence, this may mean either a mitotic stimulation or a prolongation of the prophase stage. If no increase is found, it may mean either that conditions are unchanged, or that compensating changes have taken place in the starting of new prophases and in the delivering of prophases over to metaphase. If, finally, a decrease in prophases is encountered, a toxic effect is indicated of the pre-prophasic poisoning type (D'AMATO, 1949), that is the opposite of a stimulation of mitoses. (A shortening of the duration of the prophase would have the same effect but is less probable.)

The prophase frequencies recorded are collected in Table 2. No difference is seen between groups 1 and 2, hence no stimulating effect of weak colchicine concentrations was indicated in my material. An inhibitory effect in group 3 was quite evident. Stronger concentrations evidently bring about an initial shock effect, checking the start of new mitoses during the first three hours. After that an indication of increase in prophase frequency was seen in the groups 4 and 5. As discussed above, this does not necessarily mean any stimulation but may depend on a prolongation of the prophase stage, or it may perhaps just be those cells of group 3 which were held up from starting mitosis, which are now under way and add to the regular prophase frequency.

3. THE METAPHASE BLOCKING.

The frequency of the different mitotic stages is seen in Table 3 and in the histogram of Fig. 9. The statistical variation between the 165 samples included in this material was pronounced, certain values

TABLE 2. *The frequency of prophases.*

Group No	Treatment	Tumour	Prophases/1000 cells 0	2	4	6	8	10	12	14	16	18	20	22	Total	Average
1	Controls	Ehrlich	—	1	1	5	4	—	2	1	—	—	—	—	14	8,3
		Landschütz	—	1	1	1	1	—	1	—	—	1	—	—	6	8,7
		Total	—	2	2	6	5	—	3	1	—	1	—	—	20	8,4
2	< 400	Ehrlich	—	6	10	8	9	10	5	1	1	—	—	—	50	7,7
		Landschütz	—	1	1	1	2	3	—	—	—	1	—	—	9	8,7
		Total	—	7	11	9	11	13	5	1	1	1	—	—	59	7,9
3	> 400 up to 3 hours	Ehrlich	7	9	3	—	—	—	—	—	—	—	—	—	19	1,9
		Landschütz	—	2	—	—	—	—	—	—	—	—	—	—	2	2,5
		Total	7	11	3	—	—	—	—	—	—	—	—	—	21	2,0
4	> 400 3—24 hours	Ehrlich	7	1	1	2	2	1	—	2	1	1	1	1	20	7,5
		Landschütz	—	—	1	2	—	—	1	1	—	1	2	—	8	12,8
		Total	7	1	2	4	2	1	1	3	1	2	3	1	28	9,0
5	> 400 > 24 hours	Ehrlich	1	—	2	2	6	3	2	1	2	—	1	—	20	10,0
		Landschütz	2	—	2	2	4	4	1	2	—	—	—	—	17	8,1
		Total	3	—	4	4	10	7	3	3	2	—	1	—	37	9,1

TABLE 3. *Frequency in percentages of different mitotic stages.*

Group No.	1			2			3			4			5		
Treatment	Controls			< 400			> 400 up to 3 hours			> 400 3—24 hours			> 400 > 24 hours		
Tumour	Ehrlich	Landschütz	Total	Ehrlich	Landschütz	Total	Ehrlich	Landschütz	Total	Ehrlich	Landschütz	Total	Ehrlich	Landschütz	Total
Prophases	34,2	40,0	35,9	21,9	22,3	21,9	10,4	8,7	10,3	8,1	11,7	9,1	24,1	20,9	22,6
Metaphases	41,7	43,3	42,2	55,8	66,8	57,5	73,9	87,4	75,1	66,2	83,2	71,1	51,7	63,2	57,0
Anaphases	7,3	8,0	7,5	7,3	4,6	6,9	1,4	0	1,3	2,4	0,9	2,0	9,2	5,7	7,6
Telophases	16,8	8,7	14,4	15,1	6,2	13,8	14,3	4,0	13,4	23,3	4,2	17,9	15,1	10,2	12,9
Number of cells	381	135	516	1877	370	2247	648	79	72,7	1322	902	2224	833	618	1451

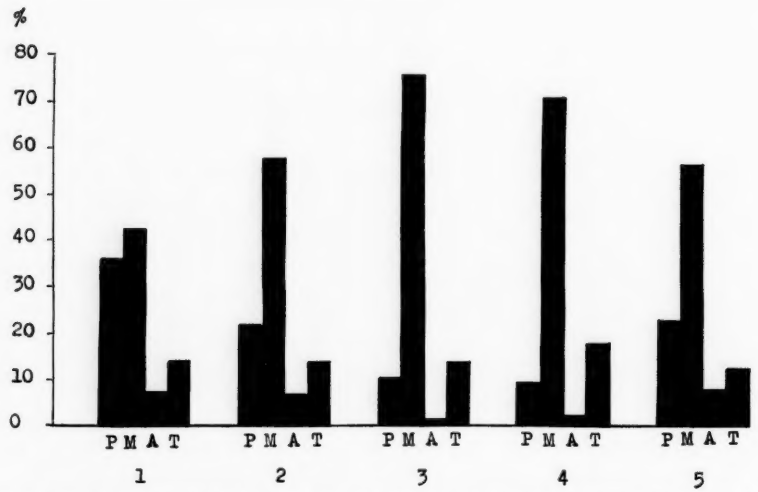


Fig. 9. The frequencies of the different mitotic stages in the five groups (1=controls; 2=weak doses; 3=strong doses, in treatments up to 3 hours; 4=strong doses, from 3 to 24 hours; 5=strong doses, treatments longer than 24 hours).

were little representative due to the low number of mitoses underlying them. The number of mitoses in the various samples varied from 8 to 174, average 43. The percentages from which the means of Table 3 are calculated are given for the metaphases in Table 4. In spite of these reservations I am of the opinion that the means of Table 3 give a fair illustration of the changes in the mitotic stages induced by the colchicine treatment.

In the controls (group 1) there is little difference in frequency between prophases and metaphases. As mentioned above, the absolute frequencies of prophases are recorded more or less subjectively; the relations between different values should be usable, however. Already weak colchicine doses (group 2) effect a decided change in the picture. The frequency of metaphases goes up in relation to the prophases. Evidently the transition to anaphase does not happen with its normal rate, a surplus of metaphases is accumulating, the metaphase blocking is a reality. This condition culminates in group 3, where 100 % c-mitosis is attained: the anaphase fraction has almost disappeared, the cells enter metaphase without being able to leave it. After a few hours treatment (group 4) an interesting increase in the telophasic frequency is encountered and a simultaneous decrease in the metaphases. Here

TABLE 4. *Distribution of metaphase percentages.*

Group No.	Treatment	Tumour	Percent metaphases 0	10	20	30	40	50	60	70	80	90	100	Number of cases	Average %
1	controls	Ehrlich	1	—	—	6	3	2	1	1	—	—		14	41,7
		Landschütz	—	—	—	2	2	2	—	—	—	—		6	43,3
		Total	1	—	—	8	5	4	1	1	—	—		20	42,2
2	< 400	Ehrlich	—	—	1	5	9	17	10	5	2	1		50	55,8
		Landschütz	—	—	—	—	—	3	3	1	2	—		9	66,8
		Total	—	—	1	5	9	20	13	6	4	1		59	57,5
3	> 400 up to 3 hours	Ehrlich	—	—	—	—	—	1	6	5	7	—		19	73,9
		Landschütz	—	—	—	—	—	—	—	—	2	—		2	87,4
		Total	—	—	—	—	—	1	6	5	9	—		21	75,1
4	> 400 3—24 hours	Ehrlich	—	—	2	1	2	2	2	3	6	2		20	66,2
		Landschütz	—	—	—	—	—	—	2	1	2	3		8	83,2
		Total	—	—	2	1	2	2	4	4	8	5		28	71,1
5	> 400 > 24 hours	Ehrlich	—	—	1	2	7	5	4	1	—	—		20	51,7
		Landschütz	—	—	—	—	2	6	5	2	1	1		17	63,2
		Total	—	—	1	2	9	11	9	3	1	1		37	57,0
Total of treated material			—	—	4	8	20	34	32	18	22	7		145	62,6

the process, illustrated in Fig. 6, has started to act: the metaphases begin to change directly into telophases. The small increase in anaphases between 3 and 4 depends mainly on the appearance of a few c-anaphases, such as depicted in Fig. 7. In group 5 again conditions are on their way towards normality: the metaphase frequency is cut down, a fair frequency of normal anaphases start to appear. In the histogram of Fig. 9 a striking similarity is seen between group 5 and 2. Long-time treatments with high concentrations produce a similar type as treatments with weak concentrations.

4. THE MITOTIC FREQUENCY.

As soon as mitosis is blocked at metaphase, while new prophases are still initiated, the total mitotic frequency of the population will necessarily increase. An accumulation to the population of cells in different mitotic stages is also one of the most striking consequences of the colchicine treatments both in plants and in animals. In extreme cases every third cell may be in mitosis; during treatments of the Ehrlich tumour with N-methyl-colchic-amide LETTRÉ, KRAPP and OCHSENSCHLÄGER obtained mitotic indices of 64,8 (*l. c.*, p. 144).

An examination of the mitotic indices of my material revealed a variation between 0,8 and 34,8. The highest values were found in group 4, where high c-mitotic percentages had been acting a few hours. Table 5 gives a survey of the mitotic indices of the entire material, while Table 7 records the actual cases of group 4. In Table 6 a comparison has been attempted with the data of the colchicine experiments of LETTRÉ, KRAPP and OCHSENSCHLÄGER (*l. c.*, Table 1, p. 143, for the controls also Table 2, p. 144). These data were arranged into three groups corresponding to my groups 1, 4 and 5.

The controls had somewhat higher indices in my material than in that of the German authors. The weak concentrations (group 2) already show some increase, quite naturally, since c-mitosis undoubtedly starts already in these concentrations. Especially the long treatments with concentrations 50—200 had several clearly increased indices, 10,7 being maximum. This value was present in three days' treatment with 50, and it coincided with the characteristic increase in c-mitotic percentage earlier mentioned.

In group 3 a very clear decrease in mitotic frequency is seen, which is a consequence of the pre-prophase poisoning in this group already discussed. It seems that anaphases and telophases may be concluded during the first few hours of a colchicine treatment, while new mitoses

TABLE 5. *Distribution of mitotic index.*

Group No.	Treatment	Tumour	Mitotic index 0	1	2	3	4	5	7,5	10	15	20	25	30	50	Number of cases	Average M. I.
1	controls	Ehrlich	—	3	6	5	—	—	—	—	—	—	—	—		14	2,56
		Landschütz	1	3	1	—	—	1	—	—	—	—	—	—		6	2,25
		Total	1	6	7	5	—	1	—	—	—	—	—	—		20	2,47
2	< 400	Ehrlich	—	3	10	19	11	6	—	1	—	—	—	—		50	3,68
		Landschütz	—	—	3	2	2	1	1	—	—	—	—	—		9	4,11
		Total	—	3	13	21	13	7	1	1	—	—	—	—		59	3,74
3	> 400 up to 3 hours	Ehrlich	—	4	4	2	—	—	—	—	—	—	—	—		19	1,91
		Landschütz	—	1	—	—	—	1	—	—	—	—	—	—		2	(3,95)
		Total	—	14	4	2	—	1	—	—	—	—	—	—		21	2,11
4	> 400 3—24 hours	Ehrlich	—	5	1	2	1	2	4	—	1	1	2	1		20	9,61
		Landschütz	—	—	1	—	1	—	1	1	—	1	1	2		8	18,79
		Total	—	5	2	2	2	2	5	1	1	2	3	3		28	12,23
5	> 400 > 24 hours	Ehrlich	—	2	3	6	4	4	—	1	—	—	—	—		20	4,39
		Landschütz	—	—	6	3	3	4	1	—	—	—	—	—		17	4,18
		Total	—	2	9	9	7	8	1	1	—	—	—	—		37	4,29

TABLE 6. *Mitotic index data of* LETTRÉ, KRAPP *and* OCHSENSCHLÄGER.

Group No.	Treatment	Mitotic index 0	1	2	3	4	5	7,5	10	15	20	25	30	40	50	Number of cases	Average M. I.
1	controls	5	24	13	5	2	—	—	—	—	—	—	—	—	—	49	1,86
4	3—24 hours	4	8	6	2	6	5	3	4	1	1	2	—	1		43	7,04
5	>24 hours	—	—	1	3	1	2	2	3	1	—	—	—	—		13	7,62

do not start until later. The first period of colchicine treatment thus means a drainage of mitoses from the tissue. In group 4 prophases again have started and in this group the greatest accumulation of mitoses is found, the mitotic indices rapidly increasing to values between 20 and 35. Table 7 shows that the maximum is attained rapidly, in 6 hours the index may change from 1,1 to 29,0, or from 2,2 to 33,0. The return to normal conditions is less precipitate, which is reflected in the clearly increased values of group 5, which resembles group 2 in the distribution of mitotic stages. In the material of LETTRÉ and co-workers group 5 even somewhat exceeded group 4. Broadly speaking, however, good agreement is present between their data and mine.

5. THE MULTINUCLEATE CONDITION.

LETTRÉ and co-workers (*l. c.*, Fig. 13, p. 148) present a graph showing how the multinucleate condition culminates a few hours after the maximum of metaphase blocking. The same sequence of events was evident in my material. Most modes of the multinucleate cell frequency occurred after a treatment of 1—2 days. For each case of mitotic index distribution of Table 7 the corresponding data for the frequency of multinucleate cells have been inserted. The regularity with which these two maxima follow each other is remarkable. In a few cases the one maximum is lacking, as, for instance, the maximum of mitotic index in the seventh case of the table, to some extent also in the eighth and ninth. Here it is likely that the sampling has missed the very maximum of the index curve. At least in the cases 8 and 9 there is a small increase indicating that a maximum may be present in the vicinity.

V. THE RECOVERY AFTER C-MITOSIS.

1. CHROMOSOME-MORPHOLOGICAL.

The morphological description of the c-mitosis of chapter III led to the stage when the c-pairs enter telophase forming small separate

TABLE 7. *Mitotic index and karyomeru*

TABLE 7. *Mitotic index and karyomery.*

Case No.	Tumour	Experiment No.	Concentration	M. I. Karyomery[1]	Time of treatment 0	½	1	2	3	6	12	24	36h	2	3	4	6 d	Maximum at
1	Ehrlich	3	400	M. I.	1,2	—	1,2	1,2	—	6,5	17,2	4,6	13,8	—	4,4	—	—	12 h
				Karyomery	0	—	0	0	—	0	1	16	9	—	1	—	—	24 h
2		2	1250	M. I.	2,1	2,0	1,2	1,6	1,4	1,4	1,6	—	—	—	—	—	—	—
				Karyomery	0	0	0	1	3	2	2	—	—	—	—	—	—	—
3		5	1250	M. I.	2,4	—	—	—	—	—	31,2	8,0	—	6,8	3,7	—	2,9	12 h
				Karyomery	0	—	—	—	—	—	0	8	—	8	1	—	2	—
4		6	1250	M. I.	3,0	—	—	—	—	20,0	9,6	3,9	3,4	5,7	3,8	3,2	—	6 h
				Karyomery	0	—	—	—	—	0	6	11	1	0	1	1	—	24 h
5		5	2500	M. I.	2,5	—	—	—	—	—	26,2	7,5	—	4,3	2,7	—	1,9	12 h
				Karyomery	0	—	—	—	—	—	0	60	—	47	14	—	13	24 h
6		6	2500	M. I.	3,7	—	—	—	—	1,1	29,0	8,2	4,8	3,3	4,3	4,0	2,7	12 h
				Karyomery	0	—	—	—	—	0	4	150	80	13	1	0	5	24 h
7		1	3750	M. I.	1,9	2,7	2,8	2,6	1,9	2,5	—	1,2	1,2	—	—	—	—	—
				Karyomery	0	0	0	4	2	3	—	20	26	—	—	—	—	36 h
8		2	12500	M. I.	2,1	1,5	1,8	1,1	1,7	1,1	—	5,7	—	6,5	—	—	—	—
				Karyomery	0	0	0	0	0	0	—	6	—	59	—	—	—	2 d
9	Landschütz	4	400	M. I.	0,8	—	—	1,6	—	8,4	—	—	2,2	4,9	5,3	—	—	—
				Karyomery	0	—	—	0	—	3	—	—	16	15	4	—	—	24 h
10		4	1250	M. I.	5,4	—	—	6,3	—	34,8	—	—	9,6	3,8	3,8	—	5,1	6 h
				Karyomery	0	—	—	1	—	4	—	—	176	90	35	—	28	36 h
11		6	1250	M. I.	2,0	—	—	—	—	14,6	23,4	4,9	4,6	5,5	3,5	2,9	3,4	12 h
				Karyomery	0	—	—	—	—	0	14	41	31	10	2	1	0	24 h
12		6	2500	M. I.	1,6	—	—	—	—	2,2	33,0	29,0	2,2	4,7	2,9	2,9	—	12 h
				Karyomery	0	—	—	—	—	0	4	130	160	11	9	1	—	36 h

[1] Number of cells with pronounced karyomery per 500 cells.

nuclei. This stage develops into the characteristic multinucleate resting stage, which is the typical final stage of c-mitosis in this materal. When I tried to catch up with the continuation of this stage, or in other words to find the first prophase in cells which had undergone c-mitosis, difficulties were at first encountered. I had expected to find numerous prophases in multinucleate cells similar to those regularly found in the multinucleate form of lymphosarcoma 6C3HED (LEVAN and HAUSCHKA, 1953 a). It turned out, however, that prophases in multinucleate cells were rare, anyhow considerably rarer than they should have been if the multinucleate interphase nuclei regularly went over into multinucleate prophases.

Experiments 4 and 5, especially designed for analysis of the recovery phenomena, solved the problem. As concentrations in these experiments 1250 and 2500 were chosen which keep the cells at full c-mitosis for twelve hours, during which period numerous post-c-mitotic cells accumulate. Fixations during the subsequent 4—6 days showed an increasing frequency of normal mitoses. If not every cell degenerates, which has undergone c-mitosis during the treatment, and all mitoses start from cells, which during the treatment remained in resting stage, the slides from this period should contain numerous samples of recovery stages after c-mitosis.

The first sign that something had happened was that the slides contained a higher frequency of chromosome-doubled cells than normally. A systematic analysis of the chromosome numbers was undertaken, and will be the subject of the next section of the present chapter. It showed that in certain slides as many as 80 % of the countable metaphases were doubled (2*s*). Since these tumours are known to contain only a rather low frequency of chromosome-doubled metaphases, certainly never exceeding 20 %, this increase in frequency must have been caused by the treatments. Chromosomal counts also gave the result that Landschütz after colchicine treatment consistently gave a much higher frequency of doubled cells than Ehrlich, which to some extent explained my failure to trace recovery mitoses in the Experiments 1—3, which exclusively had Ehrlich as material.

In Experiment 4, using Landschütz, I came across a great number of prophases representing the first post-c-mitotic prophase. At the same time the riddle was solved, why these prophases were difficult to find. It turned out that multinucleate prophases were rare, at least the extreme cases of multinuclearity which had been expected. Either the resting nuclei of each cell are capable of fusing, or the extremely multi-

nucleate interphases are not developing further. The frequency of degenerating multinucleate cells is not especially high, however, and, in addition, KLEIN, KLEIN and KLEIN (1952) gave conclusive evidence that multinucleate cells after transplantation kept their »viability and proliferative capacity» (*l. c.*, p. 488). Thus, the first alternative seems more probable. A fusion of micronuclei after colchicine treatment of regenerating liver cells was suggested by BRUES and JACKSON (1937). On sampling 18—24 hours after the treatment they still found multinucleate cells, »but in addition to these binucleate and trinucleate cells can be seen, as well as cells with single ameboid-shaped nuclei (Fig. 8), the conformation of which suggests that they may represent early fusion of micronuclei to form a single resting nucleus» (*l. c.*, p. 509). In the present case it seems that the fusions take place during interphase or at an early prophase, or possibly that the dissolution of the nuclear membrane happens so early that the prophase chromosomes already are free in the plasm, when they reach a stage where they can be observed. Furthermore, even at the earliest prophases the chromosomes of the c-pairs were as a rule free from each other, their centromeres evidently having divided during interphase. Since the chromosomes were not involved in any relational spiral, their paired arrangement was not especially pronounced.

The disappearance of this arrangement occurred unequally fast, there was no difficulty in finding traces of it, and in many cases the paired condition during late prophase was still so evident that the falling apart of the centromeres must have taken place rather late. Such stages, common in Landschütz, were also seen in Ehrlich, although more as exceptions. Generally speaking, the variation in chromosome behaviour during recovery is quite striking, chromosome shape, contraction, arrangement, cellular and chromosome size, *etc.* All these properties varied strongly. Fig. 10 may give an idea of this variation, all pictures of entire cells or chromosome plates are represented in the same scale. Evidently the colchicine treatment effects a serious disturbance of the cellular balance, resulting in the appearance of a great many abnormal cell types and chromosome types.

For the present only a small selection of the most common, or most striking, recovery variations will be described. A detailed analysis of the chromosomal variations in form and behaviour during the recovery time would have great interest. Quite possibly the detailed comparison between this variation in cancer tissue with that of normal tissue would

a
b
c
d
e
f
g
h
i
j
k
l
m
n
o
p
q
r
s
t

be rewarding (as, for instance, a study of the recovery stages in hepatoma cells compared with those of regenerating liver cells).

Fig. 10 *a*—*c* are post-c-mitotic prophases, in which the paired arrangement is still pronounced. They show a characteristic feature: the synchronization of the prophase chromosomes is upset. While most chromosomes are free out in the plasm on their way to take pro-metaphase appearance, in each cell there remains a couple of micronuclei, the chromosomes of which are in an earlier developmental stage. At first it was suspected that elements of two different cells had happened to mix during the pressing-out of the slide. This turned out not to be the case, however, since very often micronuclei of the same cell represented different stages of development. This is strictly different from the conditions in the multinucleate 6C3HED, quoted above, where the different micronuclei of one cell always had the same development. In that case it was the question of a naturally occurring variant of the tumour, which evidently had positive selection value as compared with the original type, while the present case is a matter of poisoning induced from the outside.

Also in Fig. 10 *d* there is a small group of prophase chromosomes still wrapped up in their nuclear membrane, while the rest of the chromosomes, although of highly varying spiralization, already are roughly arranged on an equatorial plate. Fig. 10 *e*—*g*, as well as Fig. 11 *g*—*i* are somewhat later prophases-prometaphases. Fig. 10 *e*, photographed as Fig. 11 *g*, is an Ehrlich cell with about half the *s* number of c-pairs. Every single c-pair in this cell recalls in shape and appearance the c-pairs during the scattered telophase. During the pro-metaphase, however, the chromosomes are divided, each c-pair now having four chromatids (Fig. 10 *h*—*m*). This division of each chromosome can be seen in some of the chromosomes of the photomicrograph of Fig. 11 *g*. These instances, so revealing for the interpretation of the post-c-mitotic division, are exceptions. Usually the division of the centromeres has occurred very early, leaving quite ordinary looking prophases to develop into quite normal metaphases. They have doubled chromosome numbers, but no arrangement in pairs any longer.

Metaphases, such as Fig. 10 *n* and *o*, are similar to those cases of

Fig. 10. Recovery stages following c-mitosis. *a*—*j*: prophase, *k*—*m*: prometaphase, *n*—*s*: metaphase, *t*: anaphase; *n*—*o*: endoreduplication type, *p*: cell with hyperhaploid chromosome number (24), chromosomes super-contracted, *q*, *r*: arrangement in c-pairs still maintained from the preceding c-mitosis, *s*, *t*: multipolar spindle. *Treatments*: *m*, *q*: 125, *h*: 2500, the rest: 1250, 1—2 days. *Material*: *e*, *l*, *m*, *q*: Ehrlich, the rest: Landschütz. — *h*—*m* ×1600, the rest ×820.

endo-reduplication recently analysed by LEVAN and HAUSCHKA (1953 b) for several ascites tumours, among them Ehrlich. It is impossible to say whether the cases here reproduced were induced by the colchicine or if they were spontaneous. It would be difficult to distinguish at late stages between diplo-chromosomes originated through endo-reduplication and diplo-chromosomes derived from c-pairs, in which the centromeres for one reason or other have remained undivided. At early stages they might be distinguished by the fact that the c-pairs would not be involved in any relational spiral, which the endo-reduplicated diplo-chromosomes are known to be.

Fig. 10 *q* and *r* are metaphases in which some of the c-pairs can still be discerned. At the same time as the c-effect disappears, the spindle starts to function. Many of the recovery stages of Fig. 10 are influenced by the spindle: Fig. 10 *d*, *n*, *q*, *r*. In Fig. 10 *s* and *t* two cells with a multipolar spindle are pictured. As is well known, such occur spontaneously in tumour tissue, but the frequency of spindle disturbances is clearly increased during recovery. Fig. 11 *f* is an instance of such a disturbance, the spindle is bipolar, but the poles are extremely broad and flattened.

Fig. 10 *p* and Fig. 11 *e* are instances of a characteristic variant occurring quite frequently under certain recovery conditions. The chromosomes are supercontracted, their breadth often exceeding their length. What is especially interesting with this chromosome type, however, is that it is correlated with a hypo-stemline chromosome number. On examining the slides of Ehrlich in Experiment 6, I made a record of the chromosome numbers of all the cells of this type encountered. They started to appear after 24 hours' treatment at about the same time as the recovery of the spindle function. At this time the multipolar spindles are frequent, thus the condition for the origin of deviating chromosome numbers is favourable. Besides the expected classes $\pm s$, $\pm 2s$ and $> 2s$ there was found from 24 hours and later a small number of cells which could not be put into these classes: (1) cells with chromosome numbers far below *s*, sometimes even below $^1/_2 s$, and (2) cells with numbers half-way between *s* and $2s$ ($2n = 95$—120). While the chromosomes of the latter group generally had normal appearance, the former group included mainly cells with the chromosome type of Fig. 10 *p* and Fig. 11 *e*. The following chromosome numbers were recorded within this group: 24, 28, 30, 32, 33, 34, 35, 37 (two cases), 38, 44, 46, 51, 52, 53, 55, and 58. In addition one cell with 50 chromosomes but normal chromosome type was found.

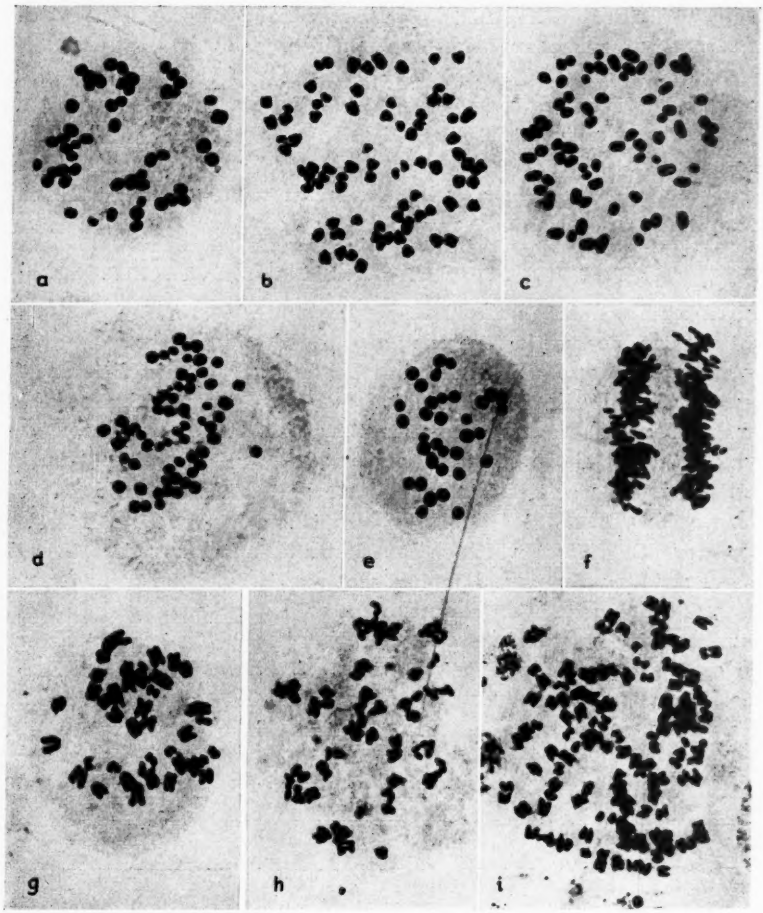


Fig. 11. Photomicrographs of *a*—*e*: scattered c-metaphases, *f*: anaphase with flattened poles, *g*—*l*: post-c-mitotic prophase (*g* same cell as in Fig. 10 *e*). — ×800.

This chromosome type was seen in Landschütz, too. It showed correlation with low chromosome number also here, although the lower limit of Ehrlich was not exceeded, 25 being the lowest chromosome number recorded in Landschütz. There is little doubt, however, that this interesting supercontracted chromosome type is correlated with low chromosome number. This does, of course, not exclude that it may be

found also in cells with higher chromosome number, or that chromosomes of normal type cannot appear in cells with low number. In human tumours KOLLER (1947) remarks that the chromosomes of cells with low chromosome numbers »were longer and more slender than in normal diploid cells» (*l. c.*, p. 46). Hypoploid cells occur normally in the ascites tumours studied (LEVAN and HAUSCHKA, 1952) but their frequency is undoubtedly much higher during the recovery period after colchicine treatment.

2. THE INDUCTION OF CHROMOSOME DOUBLING.

After Experiments 4 and 5 had yielded the interesting result that not only an increase in frequency of doubled cells was induced by the colchicine, but also a difference in reaction was suggested between the two tumours, Experiment 6 was designed for a closer analysis of these two phenomena. The same concentrations, 1250 and 2500, were chosen, which lie well above the threshold for full c-mitosis without having too much toxic side-effect. Between these two concentrations is the dosage that KLEIN (1951) used for daily treatments; the concentrations are lower than those which in LUDFORD's experiments (1936) brought about serious poisoning. Sampling was made 6, 12, 24, 36 hours, and 2, 3, 4, 6 days after the treatment. On each occasion the chromosome numbers were counted in usually 200 metaphase plates taken at random. In order to avoid any selection of the plates every plate found was classified, even if its exact count could not be determined. Three classes were recorded, s, $2s$ and $> 2s$. In all, 8166 plates were counted.

TABLE 8. *The percentage of doubled metaphases.*

Tumour		Landschütz			Ehrlich		
Concentration		1250		2500	1250	2500	
Experiment No.		4	6	6	6	5	6
Time of treatment	0	20,6	1,5	5,0	1,0	2,8	3,5
	6 h	13,7	0,5	1,5	1,5	2,7	0
	12 h	—	3,0	3,5	6,5	—	2,0
	24 h	83,7	10,5	6,4	11,8	5,8	6,3
	36 h	72,1	59,2	89,5	5,1	—	14,5
	2 d	—	27,5	82,0	5,0	32,0	29,5
	3 d	43,2	32,0	66,5	5,0	20,7	13,0
	4 d	—	24,0	52,0	2,0	11,0	4,0
	6 d	37,2	16,5	—	—	—	—

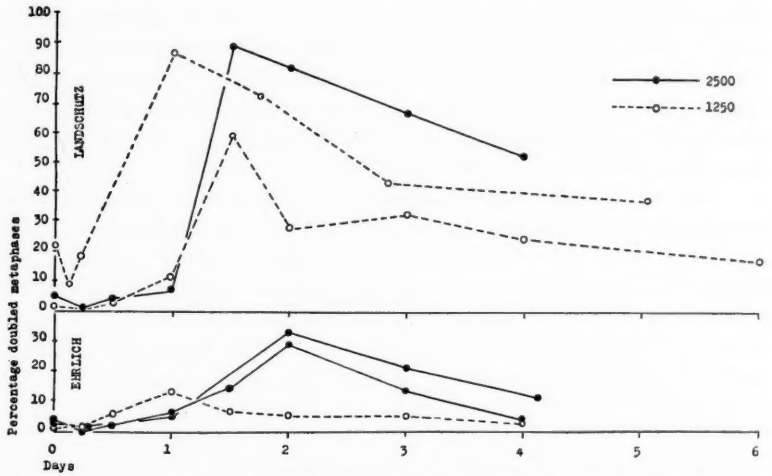


Fig. 12. Percentage doubled metaphases at certain time intervals after injection of a dose of 1250 and 2500×10^{-9} mol/kg, respectively, Upper curves Landschütz, lower curves Ehrlich.

The result was in good agreement with the orientation obtained in Experiments 4 and 5. All values of the frequency of $2s+ > 2s$ are collected in Table 8 and graphically reproduced in Fig. 12. The difference in response between Ehrlich and Landschütz comes out very clearly: in Landschütz the doublings appear earlier and their frequency reaches about three times that of Ehrlich. Maximum in Landschütz is situated at about 24—36 hours after the treatment, while the same value for Ehrlich is at 48 hours. The small maximum in the 1250 curve of Ehrlich after 24 hours is disregarded, it is certainly not clearly distinct from surrounding classes. In all cases the maximum is situated behind the maximum for the mitotic index and coincides evidently with the recovery phenomena. The maximum comprises the first mitoses following the induced c-mitosis.

The maxima of Landschütz reach 89,5 %, while those of Ehrlich stay at 32,9 %. The change brought about to the cell population of Landschütz was especially striking. After longer times of treatment, when the mitoses no longer were showing c-type, the samples gave the impression of belonging to another tumour with a hyper-tetraploid stemline. The sampling was continued during the entire life-time of the animals. During this period the $2s$ fraction showed a slow but steady decrease, somewhat more rapid in the concentration 2500 than in 1250.

The difference in reaction between the two concentrations was striking in Ehrlich, where 2500 gave a clear increase in the frequency of 2*s* cells, while 1250 hardly induced any significant increase of doublings. If this concentration had been studied only in the Ehrlich tumour, it is quite possible that the doubling action of the colchicine treatments had not been detected.

The main result of these experiments is that they conclusively demonstrate that colchicine may give rise to normal, doubled mitoses, which evidently are capable of continuing further mitotic cycles. Especially in Landschütz the frequency of 2*s* mitoses goes down so slowly that the 2*s* cells must be supposed to take part in several subsequent mitoses, even though the *s* mitoses gradually take the upper hand. As mentioned in the introduction, it has often been suspected that the behaviour of the centrosomic apparatus during c-mitosis should cause difficulties for a normal recovery process in animal material. Conclusions have been made from the behaviour of the centromeres in plant cells. It has been claimed that the centrosomes of animal cells should divide during c-mitosis, just as the centromeres of plants are known to do. In the present experiments, however, it is clearly demonstrated that this needs not necessarily be the case: normal bipolar mitoses are the rule during the recovery period, even if the frequency of multipolarity is increased (as, for that part, is the case also within plants). There is no indication that 2*s* cells, which in most cases have originated from c-mitoses, are more subject to multipolarity than *s* cells.

The difference in response between Landschütz and Ehrlich is of great interest. It may be conditioned by different circumstances. A somewhat greater sensitivity to colchicine in Landschütz was suggested by the recovery data of chapter II, Ehrlich returning to normal conditions somewhat faster than Landschütz (Fig. 4). Evidently the duration of the period for 100 % c-mitosis and the number of cells entering mitosis during this period are decisive factors. Since no differences in mitotic index were found between the tumours it may be that the period of 100 % c-mitosis is somewhat longer in Landschütz. Another factor, certainly of importance in this connection, is the relative viability of the doubling products of the two tumours. 2*s* means for Landschütz ± 92, for Ehrlich ± 160. Undoubtedly the former number has greater competitive capacity than the latter.

It is quite striking, how slowly the Landschütz population returns to normal conditions. In such circumstances the chance for the induction of a permanent change in the population should be consider-

able: it would only require a mutative change in one of the doubled cells, speeding up the mitotic rate a little; after that the tumour would shift over to 2*s* modality. Now, unfortunately, a tumour can be grown only for a limited time in the same mouse. On transplantation the conditions are radically changed, the harder antigenic situation in a new host would probably favour the old stabilized *s* cells at the cost of the still unbalanced 2*s* cells.

One small transplantation experiment was attempted (Experiment 6 *c*). On the fourth day of treatment samples taken from Landschütz were implanted into new animals. The tumours came up in 8 days, and chromosome counts on day 8, 11 and 14 gave the following percentages for doubled metaphases:

Landschütz treated with	% doubled metaphases			
	Transplantate	Day 8	Day 11	Day 14
1250	24,0	4,0	4,0	7,0
2500	52,0	19,0	15,0	11,0

There is possibly a difference between the two transplantations, indicating that the 1250 sample already at the start in the new host has restituted more or less normal conditions, while the 2500 sample still continues in the new host the process of going back to the old cell population.

It must be stressed, however, that too much significance should not be attached to these single experiments, since the frequency of 2*s* cells in Landschütz may vary even under normal conditions. It is true that I have exceptionally encountered a spontaneous frequency of 2*s* cells approaching 20 %, and since the frequency of endomitotic resting stages seems to be highly variable, it might well be expected that it may result now and then in waves of doubled mitoses. These experiments show, however, that a high frequency of doubling was effected by the colchicine, and that the cell population has a strong capacity of restituting their normal composition, in which a dynamic equilibrium is valid between the new-formation of doubled cells and their elimination due to their inferior competitive ability.

3. AN EXPERIMENT WITH REPEATED COLCHICINE TREATMENT.

Starting from the experiments of KLEIN (1951), already referred to several times, involving daily colchicine injections during the entire life time of the inoculated mouse, a preliminary experiment of a similar kind was performed (Experiment 6 *a*). Although no far-reaching conclusions can be drawn from this experiment, it nevertheless gives some

information concerning the cytological situation of such prolonged treatments. As mentioned above, KLEIN gave daily doses of colchicine, lying over the threshold for c-mitosis. This retarded but could not prevent cell multiplication, for ascites developed after 12—16 days, *i. e.* after 10—14 doses of colchicine, about which the present experiments have shown that they, given as single treatments, are followed after one hour by a twelve-hours period of practically 100 % c-mitosis, which during the next twelve-hours period does not go below 50 %. Since increase in cell number cannot take place in cells undergoing c-mitosis, we encounter here a similar problem as when in botanical material root tips continue growing in length and cell number, although they are permanently surrounded by a colchicine concentration above the c-mitotic threshold. Evidently the cancer cells respond somewhat differently to repeated treatments than to single-treatments.

The simplest explanation would have been that the cells may acquire resistance towards colchicine, KLEIN, KLEIN and KLEIN (1952) have disposed of this possibility in their case, however. An Ehrlich line which had been treated with daily colchicine injections through twelve successive transplant generations, was tested as to its colchicine sensitivity after the end of that time (the sensitivity was estimated as micronucleus production and as prolongation of the survival time). A comparison with a line which had never before been in contact with colchicine showed that no difference in reaction was present. LETTRÉ and KRAMER (1952), on the other hand, have produced, by daily treatments with N-methyl-colchic-amide through 24 transplant generations, an Ehrlich strain which tolerates a dosage of 5 γ per animal without showing any retardation in growth. »Auch Colchizin hat auf diesen Tumor keinen Einfluss» (*l. c.*, p. 117). Metaphase blocking does not occur, which indicates that the strain has acquired resistance towards the c-mitotic action of colchicine and related substances.

In the present experiment four animals, two of which had been inoculated with Ehrlich and two with Landschütz, were given daily colchicine doses of 1250 and 2500, respectively. Only one of the animals developed ascites, *viz.* the Ehrlich animal with the 1250-doses. From day 6 after inoculation attempts were made to take samples. Not until the ninth day were any cancer cells found in the samples. They were of a highly abnormal appearance, mostly giant cells with many nuclei. On day 10 (after 7 treatments) the sample contained a few tumour cells surrounded by great numbers of inflammatory cells. The tumour cells showed all signs of having undergone c-mitosis, they were larger

than normally, often multinucleate, their frequency of mitotic stages was high. Signs of defective spindle function were frequent, star-metaphases and -telophases, multipolar anaphases, *etc.* were often seen. A high frequency of 2*s* metaphases was counted, in several cases a clearly paired arrangement of the chromosomes indicated that the preceding mitosis had been a c-mitosis. In spite of all this most divisions were found to have a normal bipolar spindle. The sample had the same type as those earlier analysed with cells recovering from a 100 % c-mitotic influence.

The next day (after 8 treatments) the picture had completely changed. The abnormal giant cells were superseded by a great number of uniform cells of normal size, which were in a vivid mitotic activity. C-mitosis was rare, suggesting that the tumour had overcome the colchicine action. That this was not the case, however, was clear from the samples of the following days, when again a considerable fraction showed c-mitosis. During the days 12—14 (after 9—11 treatments) most mitoses were of c-type, mostly scattered c-metaphases and telophases. In spite of this no increase of the average chromosome number could be demonstrated. The majority of the c-mitoses during the last day had about 80 chromosomes. During the entire period a certain fraction of the cells continued developing normal bipolar spindles, the cells divided normally into daughter cells with normal chromosome number.

The experiment of KLEIN, supplemented with the present chromosome observations, leads over to a very intriguing problem, an apparent discrepancy: although c-mitosis induced by a single treatment gives rise to chromosome doubling, although a considerable percentage of the mitoses in the repeated treatments end up as scattered c-telophases, there is no indication of any increase in average chromosome number of the cell population after repeated treatments. This is found both in the DNA determinations of KLEIN and in the chromosome counts of the present paper.

As far as I can see, this situation may be interpreted along two lines: (1) The c-mitosis is modified during the prolonged treatment, so that no chromosome reproduction takes place during the resting stage following the c-mitosis. This would imply the same cytologic mechanism as functions during interkinesis, between the first and second meiotic division. This mechanism would explain, how cells could go through c-mitosis and still have their original chromosome number. It would not explain the increase in cell number evidently going on all the time. (2) The cellular reproduction is carried on by a certain fraction of the

tumour cells, which has the ability of undergoing normal mitosis in spite of the colchicine concentration, which leads most of the cells into c-mitosis.

The second alternative would show some concordance with observations made in *Allium* roots treated with supra-threshold concentrations of colchicine (LEVAN and LOTFY, 1949). It was repeatedly found that even if all older cells in the root meristems were responding with c-mitosis, certain cells of the meristems, both the tip meristem and the lateral meristems, maintained a lasting resistance towards the colchicine. There did not occur many mitoses in the very growth points, but those occurring were always normal and bipolar. They were probably responsible for the slow longitudinal growth of the roots, which remarkably enough took place even after long treatments in colchicine concentrations which to all knowledge should give 100 % c-mitoses. How this idea of differential response to colchicine of different cells may affect the interpretation of the behaviour of ascites cell populations during prolonged colchicine treatments will be briefly developed in the final section of the next chapter.

VI. DISCUSSION.

1. SURVEY OF THE RESULTS. THE THRESHOLD CONCENTRATION ZONE.

LETTRÉ was probably the first one to point out and to utilize ascites cells for the study of chemical influence on the chromosomal mechanism. The ascites cells are very favourable for such work. They constitute a kind of tissue culture *in vivo* of separately growing cells. They have many great advantages for such work: they are easy to grow, easy to influence with external agents, easy to sample, and above all, certain tumours have chromosome conditions which in clearness and surveyability compete with the most favourable plant materials.

One drawback with this technique is that dosages always will be uncertain. It is impossible to know, on injecting a substance intraperitoneally, whether it is rapidly transferred to other parts of the body and eliminated, which would make the concentration around the ascites cells lower than calculated, or if the substance is rapidly taken up by the ascites cells attaining a higher concentration in them than in the body as a whole. It has been empirically ascertained that colchicine dosages, calculated on the body weight of the animal, have been fairly reproducible. The only way out of this difficulty would be treatments

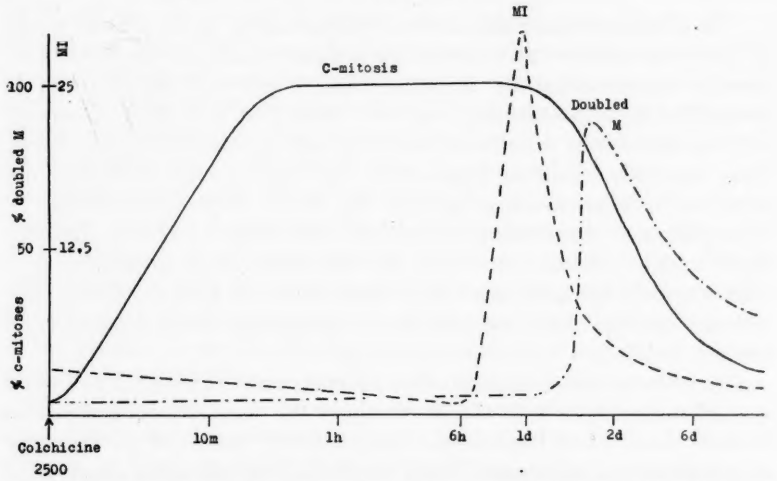


Fig. 13. The time relationships of c-mitosis, mitotic index (metaphase blocking) and doubled metaphases (post-c-mitotic metaphases).

of ascites cells grown *in vitro*. Such experiments with solid tumours (LUDFORD, 1936; TENNANT and LIEBOW, 1940) have yielded good results, although the chromosome conditions of solid tumours grown *in vitro* are not on a par with those of the best ascites tumours.

In the present investigation a rather coherent picture was obtained of the effect of colchicine on the cells and chromosomes of two ascites tumours. In Fig. 13 a somewhat schematized graph is submitted over the connection in time relationship of the c-mitosis and its main consequences. The picture refers most closely to the conditions valid after a single dose of 2500. The mitotic index curve is taken from Ehrlich, the curve of doubled metaphases from Landschütz, both out of Experiment 6, but they can be said to represent typical conditions. C-mitosis increases rapidly to 100 % during the first hour and remains at that level for some 24 hours. Mitotic index goes down at first but after 6 hours it runs upwards due to the metaphase-blocking. The curve rapidly turns and goes down again as the blocked metaphases enter telophase and interphase. After 2 days it reaches again normal level. The first recovery metaphases start to appear after one day, they quickly reach their maximum at 36 hours—2 days, whereafter they gradually go down; they will not reach normal level during the duration of the experiment.

The threshold for c-mitosis has been estimated in the present work. It was found to be situated much lower than is usual in plant material, already concentration 25 inducing clear c-mitoses. 100 % effect is attained in the concentration zone 100—400. This is in good agreement with earlier works on mouse tumours and on normal mouse tissue. Thus, the LD_{50} (median lethal dose) for male albino mice was determined by a group of workers at the Johns Hopkins University to 3,5 mg/kg, *i. e.* our concentration 8750 (GOLDBERG, ORTEGA, GOLDIN, ULLYOT and SCHOENBACH, 1950), and BERGNER (1950) in another contribution from the same laboratory determined the least colchicine dose giving c-mitotic effects on mitoses in spermatogonia as ranging from $LD_{50}/50$ to $LD_{50}/10$, *i. e.* from 175 to 875.

Colchicine treatments in other rodents make it possible to obtain a rough comparison with the size order of the threshold concentrations in these cases. Thus, BRUES and JACKSON (1937) described the influence of colchicine on rat tissues. They studied a concentration range from 20 to 250000. In the region 5000—250000 serious toxic effects prevented starting of new mitoses, in the region 250—5000 a great number of c-mitoses were found, no normal anaphases occurred, while in the lowest region 25—250 chromosome disturbances were found only in part of the mitoses. The threshold concentration is evidently of the same magnitude as in mouse ascites. On a later occasion BRUES (1951) adds some data concerning the optimal dosage for attaining metaphase blocking: »The accumulation of arrested mitoses was found to be determined very critically by both dose and time. In regenerating rat liver, the maximum percent of mitotic cells was attained between eight and ten hours after injection, with a dose of one microgram per gram body weight» (=concentration 2500). »The mitotic index fell off sharply above and below this dose ... Supralethal doses usually do not result in a metaphase count exceeding twice normal (in sarcoma 180) suggesting that the onset of prophase is inhibited» (*l. c.*, p. 1707).

In the rabbit BUCHER (1945 a, b) studied the mitotic frequency in tissue cultures of fibrocytes. He reports a metaphase-blocking with subsequent increase in mitotic index in the concentration region 41,5—62,5.

It may have some interest in this connection to examine what concentrations of colchicine were employed in the polyploidization experiments with mammals of the last years. PINCUS and WADDINGTON (1939) were using the concentration zone 500—12500 in their rabbit experiments. HÄGGQVIST and BANE (1950), also in rabbits, used the zone

4175—41750. The animal which was cytologically examined by MELANDER (1950) and was found to constitute an induced triploid originated from an inseminate containing a colchicine concentration of 5567. In swine HÄGGQVIST and BANE (1951) used the concentration 16060. As known, also here at least one triploid individual resulted (MELANDER, 1951). Thus, in all these experiments the colchicine concentrations were high above the threshold concentration for 100 % c-mitosis. BUCHER (*l. c.*) points out that the most common medical dose of colchicine, prescribed for certain forms of gout, *viz.* 1 mg, which with a body weight of 70 kg would correspond to the concentration 35,7, lies just below the concentration region, which induces increase in the mitotic frequency. LEVINE and SILVER (1947) obtained a clear increase in mitotic index in different human tumours after intramuscular injection of 2 mg colchicine. The effect culminated 16—24 hours after the injection. In human material one case is known (DUSTIN JR, 1941), in which death occurred 8 days after a dose of 60 mg colchicine taken *per os*, which would correspond to a concentration of about 2500. C-mitoses of star-type were frequent in myelocytes from sternal punctate. Also other tissues showed the typical metaphase blocking.

2. C-MITOSIS — AN ANIMAL TYPE AND A PLANT TYPE?

The course of c-mitosis as it has been described here for two ascites tumours differs from the typical behaviour in plants in one interesting point: the centromeres of the c-pairs do not divide until later, the c-anaphase is omitted, c-metaphase goes directly over into c-telophase. (In the present paper the expression »division of the centromere» at the beginning of anaphase is used to describe what is considered to be the *separation of the daughter halves* of the already divided metaphase centromere.)

This »animal» type of c-mitosis may occasionally be found in plant material after colchicine treatment or other chemical treatment. In certain colchicine experiments with plants it has even happened that c-mitosis persistently has followed the same course as here described for ascites cells. Thus, EIGSTI (1940 a, b) studying colchicine-induced c-mitosis of the pollen tube division in *Polygonatum*, *Tradescantia* and *Lilium* did not find any c-anaphase stage, the c-pairs entering the telophase nuclei without the centromeres having divided: »Daughter chromosomes have never been observed to separate completely from each other in treated pollen tubes» (1940 b, p. 520). And HAWKES (1942)

found the following c-mitotic course in *Allium Cepa*: »Contrary to LEVAN's statements the centromere of the c-pairs rarely divides until the resting stage has been entered» (*l. c.*, p. 21). As may happen also in ascites, the »normal» plant c-mitosis was occasionally found: »In a small proportion of cells and especially with some of the longer chromosomes the centromeres split before the cell enters the resting stage, but this is not at all common. When the second division under the influence of the drug is initiated all the c-pairs seem to have separated. It seems therefore that in the majority of cases the centromere splits in the resting stage or early prophase of the following division» (*l. c.*, p. 13). It would be highly interesting to try experimentally to define the factors responsible for this marked deviation from the ordinary chromosome behaviour after colchicine treatment in *Allium*. HAWKES used seedlings for his experiments, while most data on c-mitosis have been collected in roots grown on bulbs, which may perhaps make some difference.

In the ascites cells the »plant» type of c-mitosis with c-anaphase was found mainly in low concentrations close to the threshold zone. It is possible that a lower degree of poisoning favours the division of the centromere. It should be observed that there is a pronounced difference in toxicity of the colchicine between plant cells and animal cells: in plants the colchicine is comparatively non-toxic even in very strong concentrations, in *Allium* 1 % solutions do not act very toxically (that is concentrations which expressed in mol per kg $\times 10^{-9}$ would be written 25000000), and the toxicity of the threshold concentration in *Allium* is negligible, although it is some 5000 times higher than in ascites. The decrease in prophase frequency already in the upper threshold region in the ascites indicates a certain toxicity. The usual stickiness of the initial c-mitosis in ascites is also a sign of toxic action. It is quite reasonable to think that this difference in toxicity is at the base of the differential centromeric behaviour. In the ascites the toxicity may prevent the division of the centromere at the »normal» moment and have it postponed until the resting stage.

This assumption gains some support from certain experiences with plant material. It is known from treatments with various chemicals that the c-anaphase is a stage easily inhibited by toxic side-effects. C-mitosis induced by substances with more pronounced toxic action is often modified in such a manner that the c-pairs become sticky, no real c-anaphase can be found, the chromatids of the c-pairs remain associated until they are collected into a restitution nucleus. Even if the centromeres do divide this cannot be observed because of the stickiness.

This may often be the case with the more or less sticky types of ball- and bow-metaphase.

Among the by now very numerous papers on c-mitosis in the *Allium* test it happens now and then that the opinion is expressed that the term c-mitosis should be reserved to certain rather specific forms of such mitotic modifications. Morphological variations of different stages of the mitosis should disqualify it from being counted as c-mitosis. Recently, two papers (FRAHM-LELIVELD, 1949, and HINDMARSH, 1952) deal with such morphological deviations of the final stages of c-mitosis. The author of the former paper wants to reserve the term c-mitosis for the mitosis which is really polyploidogenic. In this connection she does not realize the fact that polyploid nuclei arise as a result of treatment with a great multitude of chemicals, some of which are quite simple. She separates from c-mitosis (1) tropokinesis (abnormal orientation of the spindle), (2) »mesostathmokinesis» (irregular anaphase because of stickiness), and (3) stathmokinesis (total inhibition of the spindle combined with pycnotic side-effects). In spite of the partial or total failure of the spindle characteristic of these types, she does not count them as c-mitosis. She claims that her descriptions of these types of mitotic abnormalities derive from GAVAUDAN (1943). Actually, however, his definitions are quite different, even her spelling »mesostathmokinesis» instead of merostathmokinesis is faulty. In her opinion very few agents qualify themselves as c-mitotic: »The major part of the chemicals labelled as 'polyploidogenic agents' cause the elimination of the spindle during the nuclear division when applied in concentrations more or less above their toxicity level, but this action by itself does not yet justify the term 'c-mitosis' for this phenomenon and the result is certainly not polyploidy» (*l. c.*, p. 232). By polyploidy in this connection she evidently means the production of entirely polyploid plants. Her final conclusion is then that besides colchicine hardly any more substances than acenaphthene are approved as c-mitotic, and even acenaphthene only hesitatingly. It seems strange to me that the same modification of mitosis in this way should be denoted by different terms only because in one case, due to toxicity, the number of dividing cells is small (with the result that only solitary tetraploid cells arise), while in another case the frequency of divisions is much higher (resulting in sectors or whole plants being chromosome-doubled).

HINDMARSH, too, (1952) puts up the requirement that the deviation of mitosis induced by a certain chemical must be polyploidogenic for a great fraction of cells in order to deserve the name c-mitosis. As a con-

sequence the chemicals studied by her, some nitrophenols, are rejected as c-mitotic agents, because »polyploidy, the permanent consequence of 'c-mitosis'» was not regularly observed. In addition, the author finds it dissatisfying that the centromeres, at the particular mitotic deviation induced by nitrophenols, remain undivided in blocked metaphases, no real c-anaphase being found. She recommends outmost caution in the use of the term c-mitosis: »It is essential that the term 'c-mitotic' is confined to substances which have true colchicine action» (*l. c.*, p. 158). Unfortunately her demand that the division of the centromeres at c-anaphase should be an indispensable element of c-mitosis leads to the absurd consequence that neither the c-mitosis of the present investigation, nor the c-mitosis of the pollen tube division (EIGSTI, 1940 a, b), nor the c-mitosis of the *Allium* root tips described by HAWKES (1942), should represent »true colchicine action», although they were all induced by colchicine!

In the discussed papers (FRAHM-LELIVELD, HINDMARSH) both authors are worried about the danger of using the term c-mitosis in too wide a sense: »Many authors either assume without justification that the substances which suppress spindle formation have a common mode of action, or do not recognize that by their use of this term, they imply a common mode of action for all such substances» (HINDMARSH, *l. c.*, p. 158). According to my opinion there is no need for placing any physiological implications into the term of c-mitosis. Besides I cannot consider myself responsible for the »unfortunately ambiguous» »explanation of the term 'c-mitosis'» as consisting of two parts, which HINDMARSH reads into my 1938-paper: »The first part of the explanation refers to the specific action of colchicine on the spindle mechanism, whereas the second part, and the subsequent discussion, refers to the whole cell division process» (*l. c.*, p. 160). No support for such ideas can be found in any of my papers, and the quotation made by HINDMARSH from my paper (*l. c.*, p. 160) cannot logically be interpreted in this way. However, since it would seem that such erroneous interpretations of my paper (1938) can appear, it may not be out of place briefly to summarize the origin and development of the term c-mitosis.

This term was originally introduced merely as a short designation of the mitotic deviation induced by colchicine, as studied in *Allium* roots (LEVAN, 1938). When I next (1939) compared the effect of acenaphthene with that of colchicine in the same material, I was impressed by the morphological conformity of these two reactions. Gradually more

chemicals became known to induce, broadly speaking, the same morphological effect. Even if it was realized that certain variations in the effect were characteristic of certain substances, it was also found that similar variations could be obtained with colchicine under certain conditions (other materials, other dosages). I hesitated somewhat before I included the spindle inactivation caused by inorganic salt solutions under the same term, but the perfect identity in morphology between colchicine action and the effect of certain salts of heavy metals, as mercury and lead salts, seemed to justify such a decision (LEVAN, 1945). In the c-mitosis induced by salts, however, a striking variation in type of the various elements of c-mitosis was encountered. I still found it convenient to include all these variations, often caused by the co-action of stickiness with complete or partial inactivation of the spindle, under the collective term c-mitosis. The following types may be especially mentioned: tropokinesis, merostathmokinesis (GAVAUDAN, 1943), echinus-type (BERGER and WITKUS, 1943), star-, ball-, exploded type (BARBER and CALLAN, 1943), bow-type (LEVAN, 1945), distributive type (NYBOM and KNUTSSON, 1947), segregational and reductional types (HUSKINS, 1948), rosette metaphases, compact, scattered metaphases (BERGNER, 1950), *etc.* An evaluation of different spindle disturbances and their relation to complete c-mitosis was made by ÖSTERGREN (1950).

Summarizingly it may be stated that it seems most convenient to use the term c-mitosis in a broad sense for all morphologically discernible disturbances of the spindle function irrespective of any notions as to the mode of action underlying the effects, just as the terms »toxic» or »lethal» can be used without implying any certain mode through which the poisoning or the death is brought about. It would be very futile in our present state of knowledge to try to create a c-mitotic terminology based on anything but pure similarity of morphological reactions.

HINDMARSH makes other misinterpretations, too, of papers in which I am a collaborator. It seems convenient to discuss here one of these cases, even though it belongs only indirectly to the problems of the present paper. It is the question of the c-mitosis recorded by LEVAN and TJIO (1948) after treatment with 13 concentrations of o-, m- and p-nitrophenol. HINDMARSH points out that c-mitosis was recorded in higher concentrations in our experiments than present in hers. »As no recovery experiments were cited by LEVAN and TJIO, it is likely that roots in which c-mitosis has been described were dead at the time of sampling» (*l. c.*, p. 162). Although not clearly stated, this conclusion may imply

the opinion that in recording of c-mitosis, which is a vital reaction only occurring in living cells, we have made the mistake of interpreting post-lethal changes of the chromosomes as c-mitosis. In reality, anybody reading the chapter on nitrophenols in our paper (LEVAN and TJIO, 1948, p. 474) will find that, alongside with observations on c-mitosis, there are also recorded data on the lethality, given as the time needed for turgescence to disappear from the root tips.

There is no difficulty in finding out that in certain cases c-mitosis was recorded in root tips that had lost their turgescence at the time of sampling. Thus, in p-nitrophenol c-mitosis was indicated after 4 hours' treatment for the concentrations 0,02—0,0002 mol/l, while 0,02 led to killing already during the first half-hour of the treatment, and 0,01 after 4 hours. This means that the cytological picture of these two concentrations show pre-lethal c-mitotic changes (in the case of 0,02 these changes were in fact only traces, but in 0,01 they were quite evident). The morphological picture in these cases clearly demonstrated that the changes recorded as c-mitosis were pre-lethal, although observed in cells already dead at the time of fixation.

In order to compare HINDMARSH's and our results in p-nitrophenol, which is the only mono-nitrophenol tabulated by HINDMARSH (*l. c.*, p. 159) the following data are submitted:

Concentration mol/l		0,1	0,05	0,02	0,01	0,005	0,002	0,001	0,000'5	0,000'2	0,000'1	0,000'05	0,000'02	0,000'01
Lethality	LEVAN and TJIO	Immediate		30 m	4 h	24 h	2 d	2 d	2 d	—	—	—	—	—
	HINDMARSH						24 h			—			—	
C-mitosis at four hours	LEVAN and TJIO	—	—	(+) −	±	+ (−)	+ (−)	+ (−)	±	± ±	—	—	— —	—
	HINDMARSH						+							

— (in case of lethality): no lethality during the experiment; — (in case of c-mitosis): no spindle suppression; $\frac{(+)}{-}$: traces of spindle suppression; ±: spindle suppressed in some cells; $\frac{+}{(-)}$: spindle suppressed in most cells; +: spindle suppressed in all cells.

The agreement between the two series is quite satisfactory. As pointed out by HINDMARSH, the toxicity in our experiments is lower, which is somewhat unexpected, since we used distilled water for our dilutions. This discrepancy, however, seems not to be very surprising

considering the great sources of error and the many differences in uncontrollable factors certainly present between an *Allium* test performed in Australia and in Sweden.

The deviation from ordinary plant c-mitosis of the ascites cells, which was the starting-point of the above discussion, centers around one conspicuous moment: the absence of the c-anaphase. The scarcity of detailed analyses of c-mitosis in higher animals makes it at present impossible to decide whether this deviation is typical of cancer tissue as opposed to normal tissue, or, which *a priori* would seem more likely, is characteristic also of normal tissue of higher animals in general. It may be recalled that BRUES and JACKSON (1937) describe c-mitosis in regenerating rat liver in the following way: »The arrested mitosis in the hepatic cell differs from that in most other cells (including sarcoma cells) in that the chromosomes are widely scattered throughout the cell (Fig. 3). They appear to be somewhat shortened and thickened» (*l. c.*, p. 506). In the intestinal crypts, for instance, the c-mitoses are characterized by »small, closely agglutinated chromosomes». This indicates that the factor governing the scattered type of c-mitosis is the size of the cell, in which c-mitosis occurs. In big cells, such as the ascites cells here studied, or in the liver cells, the c-mitosis takes the scattered type, while in the smaller cells of the intestinal crypts and of the solid sarcoma 180, they take an agglutinated type. Now BRUES and JACKSON continue the description of their scattered c-mitosis: »Many cells are found which are loaded with miniature nuclei, and all stages of chromosome swelling from the earliest increase in size up to the full-sized micronucleus can be observed (Fig. 7). We interpret this as indicating that the micronuclei are formed by the swelling of individual chromosomes. Where there is an agglutinated group of chromosomes they appear in similar fashion to form larger micronuclei» (*l. c.*, p. 508). Evidently the same deviation from »plant-c-mitosis» is found in liver tissue as in our ascites cells.

Some data with a bearing on this problem are also found in another group of higher animals, *viz.* Urodela. FANKHAUSER and HUMPHREY (1952) describe in the axolotl a spontaneously occurring c-mitotic mechanism leading to the origin of chromosome doublings. The division of the centromere does not take place until after the inclusion of the c-pairs in the nucleus. It seems that the chromosomes maintain their contraction until the centromeres have divided. An intra-nucleate c-anaphase may consequently be seen. The writers point out that data of a typical c-anaphase are often lacking in the cases of c-mitosis in am-

phibians. In one of these papers (PETERS, 1946), dealing with the action of colchicine on corneal cells of *Triturus*, the following is stated: »Division of the spindle-attachment region was considerably delayed. There were instances in which the chromosomes underwent reversion to interphase prior to a separation at the spindle-attachment region» (*l. c.*, p. 38).

In the present experiments it has been conclusively shown that c-mitosis in the cancer cells may lead to chromosome doubling, as ascertained in the subsequent metaphase. Although the frequency of spindle disturbances was higher in these recovery mitoses than in untreated cancer mitoses, there is no doubt that most colchicine-doubled cells gradually obtain a normally functioning spindle apparatus. It is evident that c-mitosis in mammalian cells may lead to chromosome doubling, just as in plant cells, even if the details of the c-mitotic mechanism vary. This fact is of outmost theoretical interest. Although indubitable cases of colchicine-induced doublings have been presented earlier (for instance, by PETERS, 1946, LÜSCHER, 1946 and BERGNER, 1950), considerable doubt has been raised whether such doublings are regular consequences of the c-mitosis or if they were to be considered as solitary exceptions. In the present experiments there cannot be any doubt that the induction of chromosome doubling is the regular issue of c-mitosis.

3. THE CONSEQUENCES OF C-MITOSIS FOR THE COMPOSITION OF THE CANCER CELL POPULATION.

The recovery experiments throw some light on the factors governing the composition of the cancer cell population. Identical treatment may induce a shift in its composition, different in different tumours, the decisive factor evidently being the genotype of the tumour, and other constitutional features of some permanence. Thus, in the tetraploid Ehrlich only a small shift in the direction of the 2*s* number was effected, while in the Landschütz a great proportion of cells went over to doubled condition. Whether the treatment was a single one or was repeated daily, the changed population sooner or later found its way back to the original condition. How this comes about is easily visualized as far as the one-time treatments are concerned. As soon as the colchicine concentration in the ascites cells has gone down below the c-mitotic threshold, the cells will go back to normal mitotic behaviour. It is then only necessary that those cells which have undergone no mitosis during the treatment but have managed to maintain the *s* number of chromo-

somes, divide at a somewhat higher rate than the doubled cells, in order to bring the population gradually back to the starting point. Especially in the hyperdiploid tumour this process took place rather slowly, which indicates that the difference in mitotic rate between *s* and 2*s* is not very considerable in this tumour. Only after transplantation into a new animal was the reversion to the original situation complete, or nearly so, *i. e.* the equilibrium between new-formation and elimination of 2*s* cells was attained. The restitution of normal conditions with daily treatments is more difficult to interpret. Comprehensive experiments of KLEIN, KLEIN and KLEIN (*l. c.*) have shown that this happens, however, and one preliminary experiment of the present investigation points in the same direction.

Returning to the idea that the tumour contains cells which have the ability of remaining at interphase as long as the colchicine concentration exceeds their c-mitotic threshold, but which start normal mitosis when an opportunity is furnished (in KLEIN's as well as in my repeated treatments such conditions were at hand for 25—50 % of the cells during the 12 hours preceding each treatment), it may be stated that this situation undoubtedly is in a certain accord with the conditions of root meristems. As mentioned earlier, LEVAN and LOTFY (1949) found that the youngest, least differentiated cells of such a tissue are most resistant to colchicine and are most capable of performing normal mitosis even under adverse conditions.

To what extent is it permissible to compare the cells of an ascites with those of a growing organ, such as a root tip? Even though the opinions about the cytogenetic nature of an ascites cell population may vary, it is difficult to avoid adopting some kind of a stemline concept. This means that a continuous line of cells runs through the entire development of the tumour. Just as is the case with the meristematic cells of an organ, the stemline has inherent the developmental pattern of the tumour and is responsible for the maintenance of its typical features.

Consequently the stemline cells must have a certain capacity of keeping their meristematic condition and of handing it over to at least one of the two daughter cells of each mitosis. The stemline leads directly back to the original cell (or cells), in which the malignancy once originated, therefore the stemline has started from the genotype and other permanent constitutional features of this original tissue, and is stamped by its specific characters. It is well known, however, that shifts may occur in the stemline, changing the character of the tumour. Thus,

transplantable tumours gradually lose their specific histological type, becoming more or less anaplastic. This, however, does not mean that each tumour has not still its own characteristic type. On the contrary, it is quite striking that all tumours, studied cytologically so far, have been found to be individually well-defined by such properties as the distribution of chromosome numbers, the presence of new chromosome types, the quantity and grouping of heterochromatin in the resting nuclei, the occurrence and type of doubling mechanisms, *etc.*

Many of these phenomena, as, for instance, the appearance of endomitosis in a certain fraction of the tumour cells, represent irreversible departures from the original »meristematic» condition of the stemline. Broadly speaking, each tumour follows a differentiation pattern of its own, *i. e.* »differentiated» cell types are split off from the stemline with a certain regularity. Whether this pattern is »inherited» from the tissue in which the tumour once started is a secondary question in this connection, the main point being that the stemline, growing with meristematic cells, continuously branches off »differentiated» cells.

The term »differentiated» has been put in quotation marks in order to distinguish it from the ordinary tissue differentiation, although the changes of the tumour cells are probably of similar nature; the capacity for differentiation is a general property of meristematic cells. The main difference between the stemline of a tumour and that of a normal tissue is not so much on the level of differentiation capacity as on the level of growth control; the development of a normal tissue is subjected to the correlative influence of the general developmental pattern of the organism, while the tumour stemline will grow on indefinitely and uncontrollably. Nevertheless, in both cases lines of differentiated cells branch off from the stemline according to a particular pattern, as determined by the stemline. It should be noted that in solid tumours the meristematic parts are spatially distinguished from older, more »differentiated» parts, while in ascites tumours the cells are freely movable, meristematic and »differentiated» elements are therefore intermingled. That, however, ascites tumours in their growth pattern largely obey the same rules as solid tumours has been shown recently by KLEIN and RÉVÉSZ (1953).

The shifts of tumour stemlines, now and then reported, may be looked upon as other manifestations of the differentiation process: if it so happens that during the differentiation one cell-line exceeds the stemline in viability, this line will automatically take over the stemline, with the consequence that the characters of the tumour change. The

rather regular shifts of young tumours towards more anaplastic conditions would mean that more viable cells of a less specific tissue type develop from the stemline, suppressing, or rather leaving behind, the earlier stemline cells. This is in principle the same phenomenon of cell-line selection as can be observed in any plant meristem during the recovery after a colchicine treatment: the colchicine-induced chromosome-doubled cells are, sometimes rapidly, sometimes gradually overgrown by the more »virulent» unchanged cells, still persisting in the tissue. In old, already well established transplantable tumours stemline shifts should be expected to occur more rarely; here the stemline already is the result of a lengthy »evolution» and new changes appearing will necessarily in most cases be of inferior selection value.

In all evolutionary processes the dissimilarities between permanent elements of the cell constitute the material basis for the evolution. Thus, in the colchicine-treated tissues these dissimilarities are between cells of different degree of ploidy. Genotypic differences within the germ lines constitute the basis for the evolution of organisms. The nature of significant dissimilarities underlying the evolution of tumours seems to be similar to those present at ordinary tissue differentiation, concerning the detailed mechanism of which very little is known.

While ordinary tissues and organs have a limited lifespan, the transplantable tumours have a capacity for unlimited growth in common with the germ lines of organisms. This condition has led to the peculiar situation that by now we know more about the karyological processes acting in tumours than we know about similar processes at the normal tissue differentiation. For, the longer the period is during which a stemline is growing, the more evolutionary changes will accumulate, and the further will the stemline be removed from the condition of the original hostal tissue. Thus in many cases new idiograms have been found in tumours, distinct both numerically and structurally from the idiogram of the host. The few existing data concerning the chromosomal constitution of ordinary tissues (TANAKA, 1951; HSU and POMERAT, 1953) indicate that different tissues may have specific patterns of chromosome number variation, but in no case studied has the *s* number of the tissue moved away from the $2n$ number of the animal. The capacity of the cancer stemlines to shift to new *s* modal numbers has not been found so far in normal tissue. This difference may be due to the more prolonged culture of the transplantable tumours. It is not known whether similar shifts may occur in ordinary tissues grown *in vitro* for long periods.

The capacity of developing hyperdiploid and polyploid stemlines endows the tumours with renewed and increased possibilities for experimentation with genotypical variations. Structural and other changes which would have been deleterious for a diploid stemline may be realized and compensated for in polysomatic or polyploid stemlines. The interesting situation of higher tissue compatibility found in near-tetraploid tumours as compared with diploid ones (HAUSCHKA and LEVAN, 1953 b) may thus be taken as an indication of the potentialities for increased vigour of their stemlines.

Summing up the present discussion, it may be stated that the ascites tumours can to a certain degree be compared with »a heterogeneous clonal aggregate of asexually reproducing microorganisms» (LEVAN and HAUSCHKA, 1953 b, p. 1), but they may also, and probably even better, be regarded as tissues of a specific kind. They have stemlines growing with meristematic cells in one direction and giving rise to lines of »differentiated» cells in the other direction. Returning to the experimental data of the present investigation, it is therefore not unreasonable to try to adapt to ascites tumours experiences of c-mitotic responses gained in growing root tissues.

In the two ascites tumours studied the following situation was encountered: solitary cells showed c-mitotic changes even without any treatment, and very low colchicine doses were able to induce c-mitosis in certain cells, while other cells could stand hundredfold stronger doses without responding with c-mitosis. Recalling again the observations of LEVAN and LOTFY (1949) in *Allium* root meristems, the following interpretation of these facts is suggested. The most resistant ascites cells are the »young» meristematic ones, while the more sensitive cells are the »differentiated» ones. The latter may even be of such a high sensitivity as to respond with c-mitosis, either partial (multipolarity, star mitosis) or total (scattered mitosis, »ball chromosomes»), to normally occurring metabolites, as indeed those mitotic deviations are always present in all tumours studied. The highly extended width of the c-mitotic threshold region in ascites as compared with plants would be a consequence of the differences in sensitivity of different elements of the tumour. In comparison with a root meristem a tumour would represent a considerably greater diversity of cells with different colchicine-sensitivity. I am well aware that the present interpretation is only one out of several possible ones. Thus it might be suggested that the diversity in reaction between different cells of a tumour is merely a reflection of fluctuations

in the real colchicine concentration present in different parts of the tumour, and at various times after the colchicine injection.

Even after a tumour cell has become »differentiated» and has thus deviated from the stemline, it may continue to undergo some mitoses, but compared with the stemline cells its future is limited. Some of the »differentiated» cells may be those 2*s* and 4*s* cells, always present also in untreated tumours, and some of them may be those cells which grow out without any mitoses gradually forming the giant monstrosities often encountered, but naturally there is no reason, why the »differentiation» process should always involve such drastic morphological changes. Probably most »differentiated» cells cannot be identified in the microscope. It is evident that as a cell goes off from the stemline there is no longer any necessity for the developmental power of the tumour that the mitotic mechanism of the cell is maintained intact. It is no longer of any consequence if the chromosome number changes or if structural chromosome alterations occur. The situation is parallel to those cases of normal tissue differentiation, in which cells without significance for the further development of the tissue often undergo endomitosis or even structural changes. Cases of spontaneous chromosome doubling in differentiated tissues are well-known both in plants and in animals, the classical observations being WINKLER's (1916) in *Solanum* and GEITLER's (1939) in *Gerris*. Spontaneous structural changes in old normal tissues, or in tissues without further consequence for the development of the individual, have so far been observed and analysed only in a few plants, as in cortical tissue of *Allium* roots (D'AMATO and AVANZI, 1948; LEVAN and LOTFY, 1949; THERMAN, 1951), the same tissue of *Vicia* (LEVAN and LOTFY, 1950), *Pisum* (D'AMATO, 1951), cotyledon cells of *Pinus* (TJIO and ÖSTERGREN, 1953), and in cells of young leaves of *Ginkgo* (TANAKA, TAKEMASA and SINOTO, 1952).

The tentative view of the ascites tumours suggested by the present experimental investigation implies that they exhibit cytogenetic properties both of microorganism populations and of tissues. This view has the advantage that it visualizes the coordinative principle, undoubtedly existing in the ascites, as acting not only through the differential viability of different cell types but also through a predisposition, present in the stemline, to a certain population pattern. The cytogenetic nature of each tumour is thus determined by the following three factors: (1) the acquirement of malignancy, (2) the tissue in which this change has occurred, and (3) changes taking place after the acquirement of malignancy. So far the chromosomal analysis of cancer has been able

to reach only events of group (3). On this point the chromosomal approach is helpful in different ways, as (*a*) it is of service for the taxonomy of cancer by presenting and evaluating the subtile and distinct nuclear characters; (*b*) the comparison of idiograms of different tumours with that of normal mouse tissue has indicated what may be called evolutionary principles of tumour karyotypes; and (*c*) the imitation by chemical treatment of spontaneous mitotic deviations has brought the evolutionary processes of cancer within reach of experimentation. Whether the normal tissue differentiation (2) is also connected with chromosomally seizable phenomena is an important point, still hardly touched upon. Characteristic differences in the heterochromatic situation between certain tumours, as well as the remarkable similarity in chromosome constitution in various tumours derived from similar tissue (MAKINO, 1952) are perhaps suggestive in this line. Unfortunately, the ever important problem of the malignancy (1)[1] has so far received no illumination by the chromosomal approach.

SUMMARY.

The response towards colchicine has been studied for two ascites tumours, *viz.* the hyperdiploid Landschütz I and the tetraploid Ehrlich. The threshold concentration for c-mitosis was found to be of the size order of 10^{-7} mol per 1000 g body weight, thus about 1000 times lower than the minimum concentration in which *Allium* root meristems respond with c-mitosis. The morphology of c-mitosis in the ascites cells is described. It differs from the ordinary c-mitosis of plant cells in one point: the daughter half-centromeres of each c-pair do not separate until during the resting stage following the c-mitosis.

Some accessory phenomena of c-mitosis are analysed: metaphase blocking, increased mitotic index, origin of multinucleate cells, and, during the recovery, the appearance of chromosome-doubled metaphase plates. It was demonstrated that chromosome doublings follow as a regular consequence of c-mitosis; their frequency may be high (especially in the near-diploid tumour) during the first few days following the treatment, but falls to the normal level after some time.

The last chapter has been given over to the discussion of some theoretical aspects suggested by the experiments. It is concluded that the ascites tumours, although having certain points in common with populations of asexually reproducing microorganisms, may be better thought of as tissues of specific kinds. The coordinative principle, un-

doubtedly acting in the tumours, but difficult to visualize according to the former view, becomes in the latter view an expression of the differentiation pattern inherent in the stemline of the tumour tissue.

LITERATURE CITED.

1. BACK, A. and WALASZEK, E. J. 1953. Fixation of radioactive colchicine by Ehrlich ascites carcinoma cells. — Nature 172: 202—203.
2. BARBER, H. N. and CALLAN, H. G. 1943. The effects of cold and colchicine on mitosis in the newt. — Proc. Royal Soc. B, 131: 258—271.
3. BAYREUTHER, K. 1952. Der Chromosomenbestand des Ehrlich-Ascites-Tumors der Maus. — Zschr. f. Naturforsch. 7 b: 554—557.
4. BERGER, C. A. and WITKUS, E. R. 1943. A cytological study of c-mitosis in the polysomatic plant *Spinacia oleracea*, with comparative observations on *Allium Cepa*. — Bull. Torrey Bot. Club. 70: 457—467.
5. BERGNER, A. D. 1950. Studies on colchicine derivatives. III. Effect on mitotic activity of mouse spermatogonia. — Cancer 3: 134—141.
6. BRUES, A. M. 1951. Discussion of a paper entitled »The action of colchicine on cell division in human cancer, animal, and plant tissues», by MICHAEL LEVINE. — Ann. New York Acad. Sci. 51: 1406—1408.
7. BRUES, A. M. and JACKSON, E. B. 1937. Nuclear abnormalities resulting from inhibition of mitosis by colchicine and other substances. — Amer. J. Cancer 30: 504—511.
8. BUCHER, O. 1945 a. Über die Wirkung sehr kleiner Colchicindosen nach Untersuchungen an *in vitro* gezüchteten Bindegewebszellen. — Schweizer Med. Wochenschr. 75: 715—718.
9. — 1945 b. Hemmt oder fördert Colchicin die Zellteilung? — Revue Suisse Zool. 52: 535—550.
10. D'AMATO, F. 1949. Preprophase inhibition of mitosis in root meristems. — Caryologia 1: 109—121.
11. — 1951. Mutazioni cromosomiche spontanee in plantule di *Pisum sativum* L. — Caryologia 3: 285—293.
12. D'AMATO, F. and AVANZI, M. G. 1948. Reazioni di natura auxinica ed effetti rizogeni in *Allium Cepa* L. Studio citoistologico sperimentale. — N. Giorn. Bot. It. N. S. 55: 161—213.
13. DITTMAR, C. and MAAS, H. 1944. Cytologische Studien an Tumorzellen des Mäuse-Ascites. — Zschr. f. Krebsforsch. 55: 109—134.
14. DIXON, W. E. 1905. A manual of pharmacology. — London.
15. DUSTIN JR., M. P. 1941. Intoxication mortell par la colchicine. Étude histologique et hématologique. — Bull. Acad. Roy. Méd. Belg. VI Sér. 6: 505—529.
16. DUSTIN SR., A. P. 1934. Contribution à l'étude des poisons caryoclasiques sur les tumeurs animal. II. Action de la colchicine sur le sarcome greffé, type Crocker, de la souris. — Bull. Acad. Roy. Méd. Belg. 14: 487—502.
17. EIGSTI, O. J. 1940 a. Effects of colchicine upon the nuclear and cytoplasmic phases of cell division in the pollen tube. — Genetics 25: 116—117.

18. EIGSTI, O. J. 1940 b. The effects of colchicine upon the division of the generative cell in *Polygonatum*, *Tradescantia*, and *Lilium*. — Am. Journ. Bot. 27: 512—524.
19. FANKHAUSER, G. and HUMPHREY, R. R. 1952. The rare occurrence of mitosis without spindle apparatus (»colchicine mitosis») producing endopolyploidy in embryos of the axolotl. — Proc. Nat. Acad. Sci. 38: 1073—1082.
20. FRAHM-LELIVELD, J. A. 1949. Experiments with polyploidogenic and other agents in different types of plants under tropical conditions. — Ann. Roy. Bot. Gard. Buitenzorg 51: 231—267.
21. GAVAUDAN, P. 1943. Étude quantitative de l'action mito-inhibitrice des substances aromatiques: Définition et terminologie des effets cytologiques utilisés comme test. — C. R. Soc. Biol. 137: 281—283.
22. GEITLER, L. 1939. Die Entstehung der polyploiden Somakerne der Heteropteren durch Chromosomenteilung ohne Kernteilung. — Chromosoma 1: 1—22.
23. GOLDBERG, B. *et al.* 1950. Studies on colchicine derivatives. I. Toxicity in mice and effects on mouse sarcoma 180. — Cancer 3: 124—129.
24. HAUSCHKA, T. S. and LEVAN, A. 1953. Inverse relationship between chromosome ploidy and host specificity of sixteen transplantable tumors. — Exp. Cell Res. 4: 457—467.
25. HAWKES, Y. G. 1942. Some effects of the drug colchicine on cell division. — Journ. Genetics 44: 11—22.
26. HINDMARSH, M. M. 1951. A critical consideration of c-mitosis with reference to the effects of nitrophenols. — Proc. Linnean Soc. New South Wales 76: 158—163.
27. HSU, T. C. and POMERAT, C. M. 1953. Mammalian chromosomes in vitro. III. On somatic aneuploidy. — Journ. Morph. 93: 301—330.
28. HUSKINS, C. L. 1948. Segregation and reduction in somatic tissues. — Journ. Hered. 39: 310—325.
29. HÄGGQVIST, G. and BANE, A. 1950. Chemical induction of polyploid breeds of mammals. — Kungl. Sv. Vetensk. akad. Handl. Ser. 4, 1, 12 pp.
30. — and — 1951. Kolchizininduzierte Heteroploidie beim Schwein. — Kungl. Sv. Vetensk. akad. Handl. Ser. 4, 3, 14 pp.
31. KLEIN, G. 1951. The production of ascites tumors in mice and their use in studies on some biological and chemical characteristics of neoplastic cells. — Thesis, Stockholm, 33 pp.
32. KLEIN, G., KLEIN, E. and KLEIN, E. 1952. The viability and the average desoxypentosenucleic acid content of micronuclei-containing cells produced by colchicine treatment in the Ehrlich ascites tumor. — Cancer Research 12: 484—489.
33. KLEIN, G. and RÉVÉSZ, L. 1953. Quantitative studies on the multiplication of neoplastic cells in vivo. I. Growth curves of the Ehrlich and MC1M ascites tumors. — Journ. Nat. Cancer Inst. 14. (In the press.)
34. KOLLER, P. C. 1947. Abnormal mitosis in tumours. — Brit. Journ. Cancer 1: 38—47.
35. LETTRÉ, H. 1946. Ergebnisse und Probleme der Mitosegiftforschung. — Naturwiss. 33: 75—86.
36. LETTRÉ, H. and KRAMER, W. 1952. Eine gegen Colchicin resistente Abart des Mäuse-Ascitestumors. — Naturwiss. 39: 117.
37. LETTRÉ, H., KRAPP, R. and OCHSENSCHLÄGER, M. 1950. Wirkungen des Colchi-

cins und N-Methylcolchicamids auf die Mitose der Zellen des Mäuse-Ascites-Tumors. — Zschr. f. Krebsforsch. 57: 142—150.

38. LEVAN, A. 1938. The effect of colchicine on root mitoses in *Allium*. — Hereditas XXIV: 471—486.

39. — 1940. The effect of acenaphthene and colchicine on mitosis of *Allium* and *Colchicum*. — Hereditas XXVI: 262—276.

40. — 1945. Cytological reactions induced by inorganic salt solutions. — Nature 156: 751.

41. LEVAN, A. and HAUSCHKA, T. S. 1952. Chromosome numbers of three mouse ascites tumours. — Hereditas XXXVIII: 251—255.

42. — and — 1953 a. Nuclear fragmentation — a normal feature of the mitotic cycle of lymphosarcoma cells. — Hereditas XXXIX (Festskrift för ARNE MÜNTZING 2.3.1953): 137—148.

43. — and — 1953 b. Endomitotic reduplication mechanisms in ascites tumors of the mouse. — Journ. Nat. Cancer Inst. 14: 1—43.

44. LEVAN, A. and LOTFY, T. 1949. Naphthalene acetic acid in the *Allium* test. — Hereditas XXXV: 337—374.

45. — and — 1950. Spontaneous chromosome fragmentation in seedlings of *Vicia faba*. — Hereditas XXXVI: 470—482.

46. LEVAN, A. and TJIO, J. H. 1948. Induction of chromosome fragmentation by phenols. — Hereditas XXXIV: 453—484.

47. LEVINE, M. and SILVER, G. B. 1947. Mitotic response to colchicine in human cancer. — Proc. Soc. f. Exper. Biol. Med. 65: 54—60.

48. LUDFORD, R. J. 1936. The action of toxic substances upon the division of normal and malignant cells in vitro and in vivo. — Arch f. exper. Zellforsch. 18: 411—441.

49. LÜSCHER, M. 1946. Die Entstehung polyploider Zellen durch Colchicinbehandlung im Schwanz der *Xenopus*-Larve. — Arch. JULIUS KLAUS-Stiftung 21: 303—305.

50. MAKINO, S. 1952. A cytological study of the Yoshida sarcoma, an ascites tumor of white rats. — Chromosoma 4: 649—674.

51. MELANDER, Y. 1950. Chromosome behaviour of a triploid adult rabbit as produced by HÄGGQVIST and BANE after colchicine treatment. — Hereditas XXXVI: 335—341.

52. — 1951. Polyploidy after colchicine treatment of pigs. — Hereditas XXXVII: 288—289.

53. NYBOM, N. and KNUTSSON, B. 1947. Investigations on c-mitosis in *Allium Cepa*. — Hereditas XXXIII: 220—234.

54. PETERS, J. J. 1946. A cytological study of mitosis in the cornea of *Triturus viridescens* during recovery after colchicine treatment. — Journ. exper. Zool. 133: 33—60.

55. PINCUS, G. and WADDINGTON, C. H. 1939. The effects of mitosis-inhibiting treatment on normally fertilized pre-cleave rabbit eggs. — Journ. Hered. 30: 515—518.

56. RIES, E. 1939. Die Bedeutung spezifischer Mitosegifte für allgemeinere biologische Probleme. — Naturwiss. 27: 505—515.

57. SCHAIRER, E. 1940. Der Einfluss des Colchicins auf den Mäuseasciteskrebs. — Zschr. f. Krebsforsch. 50: 143—154.

58. TANAKA, N., TAKEMASA, N. and SINOTO, Y. 1952. Karyotype analysis in Gymnospermae. I. Karyotype and chromosome bridge in the young leaf meristem of *Ginkgo biloba*. — Cytologia 17: 112—123.

59. TANAKA, T. 1951. A study of the somatic chromosomes in various organs of the white rat (*Rattus norvegicus*) especially with regard to the number and its variation (in Japanese). — Res. Genet. 2: 39—45 (cited from HSU and POMERAT, 1953).

60. TENNANT, R. and LIEBOW, A. A. 1940. The action of colchicine and of ethylcarbylamine on tissue cultures. — Yale Journ. Biol. Med. 13: 39—49.

61. THERMAN, E. 1951. The effect of indole-3-acetic acid on resting plant nuclei. 1. *Allium Cepa*. — Ann. Acad. Sci. Fenn. Ser. A. 4. 16, 40 pp.

62. TJIO, J. H. and LEVAN, A. 1954. Chromosome analysis of the Landschütz ascites tumours. — (In the press.)

63. TJIO J. H. and ÖSTERGREN, G. 1953. Spontaneous chromosome fragmentation in *Pinus*. — Proc. of the IX Congress of Genetics, Bellagio. (In the press.)

64. TSCHERMAK, E. 1943. Vergleichende und experimentelle cytologische Untersuchungen an der Gattung *Oedogonium*. — Chromosoma 2: 493—518.

65. WINKLER, H. 1916. Über die experimentelle Erzeugung von Pflanzen mit abweichenden Chromosomenzahlen. — Zschr. f. Bot. 8: 417—531.

66. ÖSTERGREN, G. 1950. Cytological standards for the quantitative estimation of spindle disturbances. — Hereditas XXXVI: 371—382.

CONTENTS.

I. Introduction. General planning of the work 1
II. Determination of the threshold concentration of c-mitosis 5
1. Earlier experiences 5
2. The present experiments 7
III. The morphology of the c-mitotic reaction in the ascites cells 13
IV. Immediate consequences of the c-mitosis 20
1. General approach 20
2. Mitotic stimulation? 23
3. The metaphase blocking 23
4. The mitotic frequency 28
5. The multinucleate condition 30
V. The recovery after c-mitosis 30
1. Chromosome-morphological 30
2. The induction of chromosome doubling 38
3. An experiment with repeated colchicine treatment 41
VI. Discussion 44
1. Survey of the results. The threshold concentration zone 44
2. C-mitosis — an animal type and a plant type? 47
3. The consequences of c-mitosis for the composition of the cancer cell population 54
Summary 60
Literature cited 61

NEUTRON AND X-RAY EXPERIMENTS IN WHEAT AND A REVISION OF THE SPELTOID PROBLEM

BY *JAMES MAC KEY*
WHEAT AND OAT DEPARTMENT, SWEDISH SEED ASSOCIATION, SVALÖF

I. INTRODUCTION.

THE artificial induction of mutation plays an important role in the study of the mechanism of heredity and evolution. During the last 25 years numerous mutation experiments have contributed to an increased knowledge of the fundamental concepts of genetics. There is, however, a pronounced disproportion in our attempt to utilize these possibilities. The diploid species have been extensively studied. Here we have an advanced knowledge of the mechanism, type and frequency of induced mutations. In polyploids, on the other hand, only comparatively few mutation experiments have been made, and none of them have aspired to more than a preliminary insight.

From these experiments, as well as from other observations, we know that the difference in mutability of polyploids and diploids is mainly based on the following conditions: (i) The reduplication of genes gives the polyploid a higher phenotypic stabilization, *i. e.*, a better buffering ability of its germ plasm (STADLER, 1929, 1930, 1931, 1932). — (ii) The higher chromosome number of the polyploid results in a higher absolute frequency of chromosome aberrations (FRÖIER, GELIN, and GUSTAFSSON, 1941; MARSHAK and BRADLEY, 1944; SMITH, 1946) and thus certainly also of other types of mutations. — (iii) The polyploid, in contrast to the diploid, is better able to bear more drastic chromosomal rearrangements without too great a loss of fertility. Thus we cannot definitely predict that the frequency of phenotypically identifiable mutations is greater in a polyploid than in a related diploid or *vice versa*. The increased number of chromosomes in polyploids works both for and against a higher observable mutability. If it is higher or lower can only be cleared up empirically.

It was in the early stage of the work on artificially induced mutations that STADLER (*l. c.*) attacked the problem. He studied the frequency of chlorophyll mutations in X_2 of different species of *Avena* and

Triticum and found the phenotypic stability due to the reduplication of genes in the polyploids to be the decisive characteristic. He thus concluded that the rate of observable mutations sharply decreases with increasing degree of polyploidy. His observation was later confirmed by MÜNTZING (1942); FRÖIER (1946); SMITH (1950), and MAC KEY (1953), and his explanation is consistent with data on the genetic constitution of the chlorophyll apparatus of oats and wheat (ÅKERMAN, 1922, 1929; SMITH and HARRINGTON, 1929; ÅKERMAN and FRÖIER, 1941; FRÖIER, 1946). STADLER's right to generalize, however, has been questioned. His statement certainly holds true for such vital factors as the ability to produce chlorophyll, where even amphidiploidy generally means a polymerization and thus a phenotypic stabilization. Experiments not restricted to the mutation rate at the seedling stage, however, have proved that a considerable frequency of off-types may be obtained in the second generation after mutagenic treatments of polyploids. This was early evident from the results on *Nicotiana* by GOODSPEED and coworkers (1928, 1929, 1930, 1937, *etc.*), and experiments with the same species that STADLER used, soon strengthened the criticism. None of the tests, however, attempted an exact evaluation nor a definite rejection of STADLER's statement.

For practical reasons most of these additional mutation experiments on polyploids have been performed on the 6*x*-wheat. Attempts to produce mutations in this plant by X-rays have been made not only by STADLER (*l. c.*) but also by DELAUNAY (1930, 1931, 1932, 1934), SAPEHIN (1930, 1934, 1935), FONDARD and CABASSON (1939), RANJAN (1940, 1946), GUSTAFSSON (1941), FRÖIER (1946), SMITH (1950), OLTMANN (1950), MAC KEY (1952), ARNASON, PERSON and NAYLOR (1952), and ONUFRIJCHUK (1953). Further, short waves have been tried by FONDARD and CABASSON (*l. c.*), electric current and centrifuging by BYNOV (1938 a and b), atomic bomb radiations by SMITH (*l. c.*), fast neutrons by MAC KEY (*l. c.*), and radiophosphorus by ARNASON, PERSON and NAYLOR (*l. c.*). Even temperature shocks (ROHWEDER, 1929; quoted from DIX, 1934; SCHKWARNIKOW, 1936), storing of the seed for 7—10 years (SCHKWARNIKOW, 1937, 1939), and colchicine treatment (PIROVANO, 1939) were used with some success to increase the mutation frequency. Also the attempts of DIX (1934) may be mentioned, when he tried to induce mutations in the meristem of wheat seedlings just starting to tiller. In addition to X-rays and ultraviolet radiation, he tested 5 and 10 per cent salt, nitric and acetic acid, sodium and ammonium hydroxide, ether and chloroform.

In many cases the cited experiments have been very preliminary without controls nor exact doses. Seedlings or gametes have often been treated, as these require lower shock effects than the seed. Such experiments give, however, a very low reproductivity and are, therefore, of little value when studying the response to mutagens of different varieties or species. Strong mutagens, *e. g.* X-rays, have always been able to give an appreciable frequency of mutations, if the material has only been followed long enough. In certain analyses this frequency has to be calculated as high as to 70—80 per cent of the surviving X_1 plants.

The present study is mainly concerned in developing this problem of mutability at the diploid and polyploid levels. For practical reasons common wheat ($6x=42$) was chosen as the polyploid and barley ($2x=14$) as the diploid representative. The two subjects were treated as dry seeds in the same way and analysed according to the same system. Experiments showed that the well-studied diploid barley has the same genetic response to mutagens as einkorn-wheat (MAC KEY, unpubl.; *cf.* also FRÖIER, 1946). A direct comparison of the mutability in diploid barley and hexaploid wheat thus seems justifiable. Just how wide a generalization can be made from the present findings is, however, a more delicate question.

In order to make the analysis as general as possible, it was considered inadvisable to stick to one mutagen only. Therefore X-rays, fast neutrons and nitrogen mustard were included in the test. These mutagens were chosen because neutrons were known to induce a high proportion of chromosome alterations, while mustards were found to give comparatively more point mutations; X-rays, on the other hand, were the old standard of intermediate effect (*cf.* LEA, 1946; AUERBACH, 1949; CATCHESIDE, 1948). With this choice of mutagens it was felt that the possibilities of establishing whether chromosomal rearrangements or point mutations were of greatest value to plant breeding were considerably increased. Here one simply could not expect the answer to be identical for a diploid and a polyploid.

Even if this broad attack on the problem of mutability proved very fortunate, the whole program could not be carried out. The nitrogen mustard showed too high a toxicity and too low a mutagenic effect on the seed to justify a more profound analysis. The results on this point are briefly reported in a recent paper (MAC KEY, 1954 a), while the interest this time is totally concentrated on the results with X-rays and fast neutrons. Further, the barley experiments will only be discussed here as a basis of comparison with the wheat experiments, since the

first phases of this work on barley have already been reported in detail elsewhere (MAC KEY, 1951).

The mutation experiments on hexaploid wheat have passed the stage of the progeny test, but the genetical and cytological analyses of the individual types of mutation are far from finished. Up till now the main interest has been concentrated on the speltoid and compactoid mutations, since these made possible a stimulating study of the mutagenic action on a single chromosome.

II. MATERIAL AND METHODS.

The results presented in this paper are in the main based on three series of experiments. They were initiated by Dr. K. FRÖIER in 1943, and by the author in 1948 and 1949. The first one only included X-radiation of a purified stock of Svalöf winter wheat Scandia III A, in the year of treatment brought up to F_{20}. In the two later experiments, where both fast neutrons and X-rays were applied, spring wheat was chosen. The spring and winter habit proved to influence the radiosensitivity (MAC KEY, 1954 c) and a direct comparison with the extensive study on the diploid spring barley was desired. A strain of Svalöf spring wheat Rival, subjected to repeated individual selection, and brought up to F_{24} and F_{25} in the years of irradiation, was chosen as the most suitable material. The two varieties belong to *Triticum vulgare* var. *lutescens* AL.[1] and are bred and described by ÅKERMAN (1944, 1951). The irradiated dry seed of Scandia III was sieved ($>$ 2,50 mm) and had a water content of 19,4 per cent. Since FRÖIER and GUSTAFSSON in 1944 were able to show a correlation between seed size and radiosensitivity, the succeeding experiments with spring wheat were based on still more uniform seeds of the sieve fraction 2,25—2,50 mm. GUSTAFSSON (1947) showed that even slight differences in the water content of the irradiated seed may influence its sensitivity and thus the reproductivity of the experiment, and for this reason the water content was fixed at a low uniform level, 10,4 per cent, in the two experiments with spring wheat. Last year's harvest was used as seed in all the three series.

[1] According to the Int. Code of Bot. Nomenclature (Utrecht 1952), the name *Triticum vulgare* HOST should be substituted by the older *Tr. aestivum* L. (*cf.* also SCHIEMANN, 1949). It is, however, the opinion of the present writer that the hexaploid wheats comprise one species only, and this must be called *Tr. aestivum L. em.* THELL., while the common bread wheat should be named *Tr. aestivum* L. *em.* THELL. ssp. *vulgare* (VILL., HOST) *n. comb.*, the spelt wheat ssp. *spelta* (L.) THELL., and the club wheat ssp. *compactum* (HOST) *n. comb.* A detailed argumentation will be published in the near future. While waiting, the old names are here consistently retained.

The first generation, here denoted by X_1 for X-ray and N_1 for neutron treatment, was more accurately handled in the spring wheat series. A maximum number of plants from the irradiated field-sown seeds were analysed after each treatment as to the proportion of fertile and sterile flowers. This fertility analysis was based on the first and second flowers only of each spikelet, since flowers of a higher order are often normally sterile and thus diminish the reproductivity of such a test. Further, every head was analysed separately and at the sowing of X_2 and N_2 it was used as the unit. This method has definite advantages when analysing the results and in addition it gives a valuable control as to whether the observed off-types are true mutations or mere products of outcrossing. In the latter case all head progenies of one plant should include the recessive off-type, while such a state is extremely rare in the first case due to the chimerical character of a mutated X_1 or N_1 plant. For financial reasons not more than 3 heads for every first generation plant were sown for further analysis, however. The experiment of 1943 with winter wheat was treated more summarily. When this was kindly put at the author's disposal as unsorted X_2 material, it was reduced to include only the control and the material irradiated by 20.000 *r*. A rough estimate of the X_1 fertility was based on the total number of grains per X_1 plant and the X_2 material was sown as plant progenies. Data from these analyses were published by FRÖIER (1946).

The progeny and segregation tests in the third and following generations were based without exception on space-planted plant progeny plots.

All cytological examinations were made on permanent smears, originally fixed in Carnoy (6 : 3 : 1), stained in Feulgen and prepared with a technique similar to that of HILLARY (1940).

The dosage of irradiation should only be briefly described here, as detailed information is already given by MAC KEY (1951).

All X-ray treatments were performed at the Radiophysics Institute, Stockholm, thanks to the kind cooperation of Dr. A. FORSSBERG. The X-rays were produced by a Coolidge type X-ray tube (Philips Metalix). The beam was filtered through 1 mm Al. The following data apply to the three series of experiments:

Year	mA	kV	Intensity r/min
1943	4	176	870
1948	6	170	1620
1949	6	170	1500

The fast neutrons were obtained from the 80 cm cyclotron of the Nobel Institute for Physics, Stockholm, by kind permission of Professor

M. SIEGBAHN and with the indispensable assistance of Dr. A. HEDGRAN. The neutrons were produced by bombarding a beryllium target with deuterons accelerated to 6,5—7 MeV, and the beam from the target was regulated to give an ionization current of $0{,}6 \cdot 10^{-7}$ A in a boron chamber. During the treatment, the seeds were put in a glass tube which was fixed in a wooden plate between the magnet poles but outside the tank and at a distance of 20 cm from the target. The glass tube was covered with a 2 mm Pb plate in order to exclude most of the γ-radiation from the cyclotron. The time of exposure ranged from 10 to 90 minutes according to the size of the dose.

Owing to the inherent difficulties in obtaining a constant beam from a cyclotron and the high dosages applied, a dose integration method by the activation of an indicator was preferred to direct measurement by means of an Aerion condenser chamber or a Victoreen *r*-dosimeter as used in most experiments of a similar nature. With the range of doses and technique of irradiation applied in the present study, the reaction $Al^{27}(n, \alpha)Na^{24}$ (14,9 hours) was found to be the most convenient one. A ring of pure Al was fixed around the middle of each glass tube during irradiation and after 6—7 hours the intensity of the Na^{24} decay ($Na^{24} \rightarrow Mg^{24} + e^- + 2\gamma$) was determined by an arrangement for β-γ-coincidence measurements (HEDGRAN, 1948). As the diameter of the glass tubes and of the Al rings conformed to that of the GM tube, these measurements could be made with high accuracy. The favourable disintegration scheme of Na^{24} allowed the number of decayed atoms per gram Al per minute to be calculated. This number, given for the middle of the exposure time, is used as a measure of the dose applied. This new unit, disintegrations per gram Al per minute (abbreviated to *dis*), gives a good reproductivity for neutrons of about the same energy as those used here. The relation between the X-ray unit *r* and the neutron unit *dis* is about 1 : 35, if that absorption of energy is considered which occurs in embryos of dormant seeds with 10 per cent water (EHRENBERG and NYBOM, 1952).

III. EFFECTS OF RADIATION IN THE FIRST GENERATION.

1. THE DIFFERENTIAL RESPONSE TO X-RAYS AND FAST NEUTRONS.

The energy absorption in tissue follows different patterns with X-ray and neutron irradiation. This depends on the different physical

properties of the ionizing particles produced. Fast neutrons dissipate their energy in biological material mainly by the production of protons, while X-rays eject electrons. The mean linear ion density in tissue is generally higher for protons than for electrons; with the radiation sources used in the present experiments it is about 4 times higher (*cf.* GRAY, 1950). Further, the protons are ejected in approximately straight lines with a rather even distribution of ions along their paths, while the electron tracks are definitely shorter, tortuous, and with the ions produced more in clusters. This gives the electrons a varying efficiency along their way through the tissue. Physically, primary ionization and excitation produced by a proton or electron of the same speed are practically indistinguishable. Thus the different biological action of X-rays and neutrons at equal energy dissipation lies in the distribution in space of ionizations and excitations which the two radiations ultimately produce.

This difference agrees very well with the biological effects of the two radiations. Where a single or a very few ionizations are needed to produce an event, X-rays are more effective than neutrons which here mean a waste of energy. An example of this are the experiments on induced recessive lethals of *Drosophila*. This type of mutation generally seems to be produced by direct ionization in the gene (*cf.* CATCHESIDE, 1948), *i. e.* small amounts of energy are needed. All experiments on recessive lethals have also shown X-rays to be more effective than neutrons in comparable doses (TIMOFÉEFF-RESSOVSKY and ZIMMER, 1938; ZIMMER and TIMOFÉEFF-RESSOVSKY, 1938; DEMPSTER, 1941; GILES, 1943; FANO, 1944). To break a chromosome, a higher concentration of energy is necessary, however, as a rule about 20 ionizations (LEA and CATCHESIDE, 1942). For this biological event, neutrons also are definitely more effective than X-rays (MARSHAK, 1939; GILES, 1940, 1943; DEMPSTER, 1941; THODAY, 1942; FANO, 1943, 1944).

The ability to induce various genetical alterations is accompanied by effects on the metabolism of the cell, often with lethal consequences. According to MAC KEY (1951, 1952) and later to other members of the Swedish research group dealing with mutation experiments (EHRENBERG *et al.*, 1952, 1953; NYBOM *et al.*, 1952) as well as to CALDECOTT, FROLIK and MORRIS (1952), these physiological reactions play a rather subordinate role with neutron treatment, while they are responsible to a high extent for the induced lethality with X-irradiation. Here, too; this difference in the biological action of the two radiations is in full accord with the different ion density characteristic to them. Where activated

water or other substances able to transfer energy are of great importance for the induction of a biological effect of the irradiation, which seems valid for many physiological reactions, too concentrated a spatial production of ions means a waste of energy. Due to the high ion concentration, reversible processes will rapidly neutralize the primary effect (*cf.* WEISS, 1944; LEA, 1946; ALLSOPP, 1948).

These earlier experiences of the different biological actions of X-rays and neutrons are verified also by the present experiments. The data which show this dissimilarity are given in Table 1. Parallel experiments with the diploid barley are also included there for comparison. For both wheat and barley, the lethality is highest after X-irradiation but the surviving individuals are always characterized here by a comparatively normal vitality. If this is measured by the tillering capacity or by the number of spikelets per head, values even higher than for the controls are obtained with more appreciable doses. This circumstance cannot, however, be interpreted as some kind of stimulation. Nor can GUSTAFSSON's (1947) explanation be accepted that the negative correlation between applied dose and number of spikelets per head is due to a systematic selection caused by the different radiosensitivity of small and large grains. Counter-evidences for this are the results of the neutron experiments as well as the fact that the seed used in the present examination was extremely uniform (barley, sieve fraction: 2,50—2,75 mm; wheat: 2,25—2,50 mm). The explanation is surely to be found in the better nutrition and light conditions due to a very thin stand. As far as the third given measure on vitality, the plant fertility, is concerned, it is certainly lower in all X-rayed material than in the controls (the values for wheat in 1948 are less representative due to an attack of wheat midge, *Contarinia tritici*). The fertility, however, does not fall proportionally with increased doses, it reaches a minimum rather quickly. This must mean that a counter-selection prevents the appearance of the more gross genetical disturbances of the surviving plants.

The effect of the neutrons is not the same. The lethality of the seedlings is here definitely lower but all plants grown up look weak and unhealthy when sufficiently high doses have been applied. This reaction is indicated by the tillering capacity as well as by the number of spikelets per head but is especially obvious from the sharply declining fertility. As expected, the last character which, according to GELIN (1941), reflects very well the degree of chromosomal disturbances, was

TABLE 1. *The response in the first generation of barley and wheat to X-rays and fast neutrons.*

Year	Treatment	No. of grains sown	No. of mature pl., %	Mean No. of spikes per plant	Mean No. of spikelets per head	Mean plant fertility, %
	Barley (2x = 14):					
1948	Control	600	91,8	4,1 ± 0,09	24,9 ± 0,10	97,0 ± 0,10
1949	»	750	91,6	4,2 ± 0,11	26,3 ± 0,10	96,1 ± 0,13
1948	10.000 *r*	450	80,9	3,7 ± 0,14	24,7 ± 0,18	74,6 ± 1,39
»	15.000 »	600	60,3	4,1 ± 0,20	24,8 ± 0,17	70,6 ± 1,35
»	20.000 »	600	43,5	5,0 ± 0,27	24,8 ± 0,15	66,6 ± 1,32
1949	5.000 »	300	91,3	3,3 ± 0,14	26,1 ± 0,19	90,8 ± 0,81
»	10.000 »	450	90,4	3,4 ± 0,18	25,1 ± 0,29	75,6 ± 1,81
»	15.000 »	600	40,8	4,1 ± 0,22	26,8 ± 0,24	77,0 ± 2,06
»	20.000 »	750	26,7	6,5 ± 0,30	29,1 ± 0,14	77,3 ± 1,94
1948	1.000 *dis*	300	91,3	3,6 ± 0,13	24,0 ± 0,18	86,2 ± 0,66
»	1.300 »	300	89,0	3,6 ± 0,13	24,0 ± 0,17	80,1 ± 0,81
»	3.300 »	600	81,5	3,6 ± 0,12	24,0 ± 0,17	54,5 ± 1,18
»	5.700 »	450	84,5	3,1 ± 0,10	23,3 ± 0,18	54,6 ± 1,12
»	6.100 »	600	85,5	4,0 ± 0,15	22,4 ± 0,18	47,0 ± 1,24
1949	1.500 »	300	94,3	4,2 ± 0,20	25,9 ± 0,21	85,4 ± 0,94
»	4.000 »	300	93,7	3,7 ± 0,15	25,2 ± 0,23	68,4 ± 1,38
»	6.400 »	300	89,7	3,0 ± 0,11	23,6 ± 0,26	56,2 ± 1,84
»	10.400 »	450	89,8	3,4 ± 0,14	24,5 ± 0,27	37,8 ± 1,54
»	14.200 »	600	79,8	3,5 ± 0,16	26,0 ± 0,27	31,1 ± 1,48
	Wheat (6x = 42):					
1948	Control	300	91,7	3,2 ± 0,09	12,9 ± 0,09	71,6 ± 0,61
1949	»	300	83,0	3,1 ± 0,10	15,6 ± 0,09	82,6 ± 0,49
1948	5.000 *r*	300	83,7	3,0 ± 0,10	13,2 ± 0,09	58,0 ± 0,84
»	10.000 »	450	75,1	2,9 ± 0,11	13,0 ± 0,10	67,1 ± 0,98
»	15.000 »	450	41,1	3,5 ± 0,18	14,7 ± 0,11	60,2 ± 1,09
»	20.000 »	600	22,2	5,2 ± 0,25	15,8 ± 0,10	75,1 ± 0,95
»	25.000 »	600	16,2	5,3 ± 0,49	16,3 ± 0,11	67,3 ± 1,04
1949	5.000 »	300	65,7	2,5 ± 0,10	14,4 ± 0,15	86,9 ± 0,62
»	10.000 »	300	18,3	4,4 ± 0,33	16,6 ± 0,14	86,6 ± 1,09
»	15.000 »	600	14,2	5,7 ± 0,27	17,1 ± 0,10	84,2 ± 0,79
»	20.000 »	600	12,0	6,8 ± 0,43	17,2 ± 0,09	84,9 ± 0,74
»	25.000 »	750	10,7	6,7 ± 0,31	17,2 ± 0,09	81,4 ± 0,92
1948	2.100 *dis*	300	91,3	2,9 ± 0,08	12,8 ± 0,09	57,3 ± 0,81
»	3.200 »	300	91,7	3,0 ± 0,07	12,5 ± 0,09	65,7 ± 0,79
»	3.300 »	450	90,9	2,9 ± 0,10	12,6 ± 0,10	64,3 ± 0,73
»	5.700 »	600	82,5	2,8 ± 0,07	10,9 ± 0,09	51,6 ± 0,93
»	8.600 »	600	87,2	3,0 ± 0,09	11,6 ± 0,10	49,8 ± 0,90
1949	2.900 »	300	82,0	2,7 ± 0,12	14,6 ± 0,11	76,5 ± 0,87
»	5.800 »	300	85,0	2,8 ± 0,10	13,7 ± 0,11	79,6 ± 0,67
»	10.600 »	450	85,1	2,7 ± 0,09	11,9 ± 0,15	72,1 ± 1,17
»	16.800 »	450	56,9	3,4 ± 0,18	12,1 ± 0,13	62,9 ± 1,37

less influenced in the polyploid than in the diploid, in full accord with the results of MÜNTZING (1941) on $2x$- and $4x$-barley.

This sharp difference in the response of seeds to X-rays and neutrons can hardly be explained simply by the hit theory. The primary effects of the irradiation cannot be entirely of chromosomal origin. If, however, it is partly attributed to a genetic action, mainly resulting in chromosome rearrangements, where neutrons are more effective, and also to a physiological non-genetic action which with X-irradiation sooner reaches fatal consequences for the cell, then the data of Table 1 will have their explanation. The difference between X-rays and neutrons in the influence on survival and vitality is natural under such conditions. Similarly, the statement verified here, *i. e.*, that the fertility of a polyploid is less affected by mutagens than that of a diploid, may be connected with the fact that the survival curve of X_1 falls steeper for the hexaploid wheat than for the diploid barley. The reduplication of genes is not able to buffer, if the lethality of X-rays is mainly of a physiological character. That the sensibility is even greater in wheat may at least partly be attributed to the fact that the barley grains are hulled, whereas in wheat they are not (*cf.* FRÖIER and GUSTAFSSON, 1944).

2. MORPHOLOGICAL ABNORMALITIES IN THE FIRST GENERATION.

In all attempts to induce mutations in the seed or the seedling, it must be borne in mind that the treated object is composed of a large number of cells. The induced changes will not leave their mark on the entire adult plant, they will give it a more or less complicated chimerical structure. If dominant mutations appear, they may thus give rise to phenotypically identifiable chimeras. However, none of the above-mentioned workers who experimented with irradiated seeds or seedlings of wheat have reported any morphological chimeras in X_1. Only RANJAN (1940) says he found some undefined abnormalities in the heads. This negative observation clearly expressed by at least STADLER (1929) and SMITH (1950) is not in conformity with the high frequency of speltoids often observed in X_2 and the knowledge about the mode of expression of this type of mutation. Even before X-irradiations of wheat were made, ÅKERMAN (1920, 1927) clearly showed that the speltoid chimeras were recognizable. For that reason a thorough analysis of the first generations of the 1948 and 1949 experiments on spring wheat was made. As shown by Table 2, which gives the total number of induced speltoid chimeras as well as some other abnormalities, this analysis has revealed a very high frequency.

TABLE 2. *Morphological abnormalities induced in the first generation of spring wheat (Rival) by X-rays and fast neutrons.*

Year	Treatment	No. of plants examined	No. of speltoid				No. of plants with			
			heterozygotes		chimeras		adventitious spikelets		bifurcated heads	
			abs.	%	abs.	%	abs.	%	abs.	%
1948	Control	275	—	0,0	—	0,0	—	0,0	—	0,0
1949	»	249	—	0,0	—	0,0	—	0,0	—	0,0
	Total	524	—	0,0	—	0,0	—	0,0	—	0,0
1948	X-rays	1004	—	0,0	22	2,2	1	0,1	2	0,2
1949	»	489	—	0,0	43	8,8	2	0,4	—	0,0
	Total	1493	—	0,0	65	4,4	3	0,2	2	0,1
1948	Neutrons	1946	1	0,1	253	13,0	10	0,5	1	0,1
1949	»	1140	3	0,3	204	17,9	5	0,4	1	0,1
	Total	3086	4	0,1	457	14,8	15	0,5	2	0,1

A test of heterogeneity performed after a correction for discontinuity and elaborated for 2×2 tables (*cf.* BONNIER and TEDIN, 1940) shows that X-irradiation definitely induces a high frequency of speltoid chimeras if compared with the untreated control ($\chi^2=22{,}20$; $P<0{,}001$). The same result is obtained by neutron treatment ($\chi^2=87{,}51$; $P<0{,}001$). Knowing that the speltoid mutation generally is induced by chromosome breaks, that these are more effectively produced by neutrons than by X-rays, and that the genetic action of X-rays is partly removed by the above-mentioned counter-selection, it seems quite natural that neutrons are able to give a higher frequency of chimeras than do X-rays ($\chi^2=107{,}86$; $P<0{,}001$). In reality, the efficiency of the neutrons is decidedly greater than Table 2 is able to show, since the dose range of the neutrons was definitely lower than that of the X-rays, if compared on the basis of equal absorption of energy.

NYBOM (unpubl. data; *cf.* MAC KEY, 1951) has calculated the relative efficiency of fast neutrons and X-rays in producing cytological disturbances in the first mitotic cycle after the germination of irradiated barley seeds to be $dis/r \simeq 0{,}6$. If this interrelation is also accepted for wheat, Fig. 1 will indicate the ability of the two radiations to induce speltoid chimeras in X_1 and N_1 with successively increasing doses. Besides the higher efficiency of the neutrons to produce this special effect, it should be pointed out that the frequency curves correspond very well to those obtained for the mutations of the second generation (*cf.* Fig. 17).

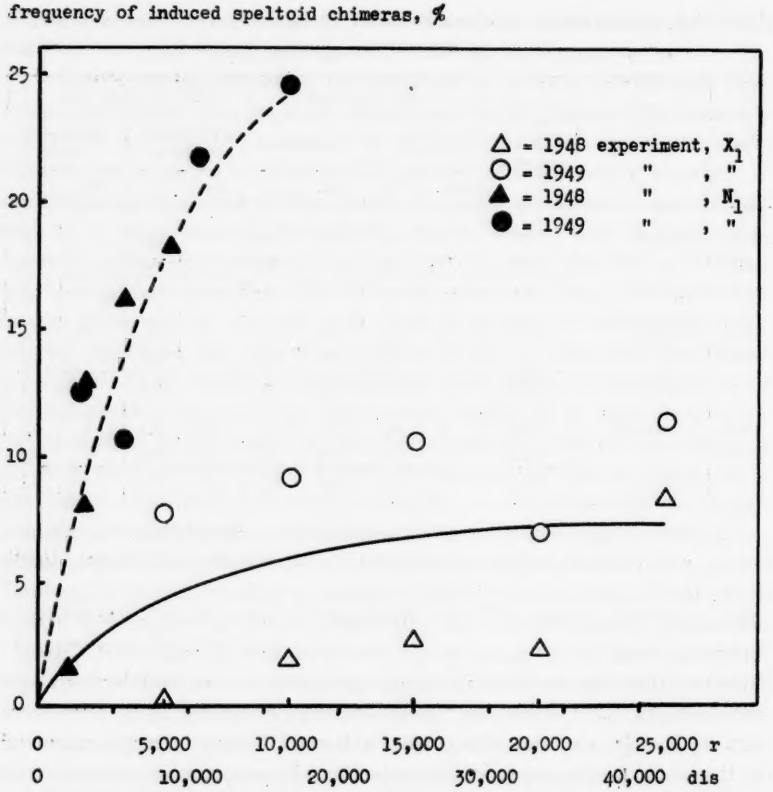


Fig. 1. The frequency of speltoid chimeras induced in the first generation from seeds of spring wheat (Rival), irradiated by successively increasing doses of X-rays and fast neutrons. Inter-radiational dose scale: 1 *dis*=0,6 *r*.

The counter-selection characteristic to X-rays gives a flat curve and a greater interannual variation, since this side of the action is more influenced by environmental conditions than the pure genetic one. Thus, the speltoid mutation is excellent to use in a rapid verification of mutagenic effects.

The high frequency of speltoid chimeras in X_1 and N_1 supports ÅKERMAN's (1920, 1927) opinion that they are produced as somatic mutations in *vulgare* tissue. Contrary to this interpretation another was introduced by KAJANUS (1923) and NILSSON-LEISSNER (1925). They claimed that the chimeras originally were heterozygous speltoids, and

that tissue of *vulgare* type had occurred through somatic segregation. The discussion could never be settled, as all speltoid chimeras known were spontaneous and for that reason the primary constitution could not be proved. The present observation, together with the finding that speltoids are mostly, if not entirely, loss mutations, solves the problem.

The study of the induced speltoid chimeras also gives some histogenetic aspects. From anatomical investigations we know that the growing tip of gramineous plants is composed of four layers of which only three are independent (for literature, see SHARMAN, 1945). This differentiation, however, seems to happen rather late. Thus, RÖSLER (1928) examined the vegetation point of young seedlings and found that the meristem of these early so-called plastocrones only has two independent layers, denoted dermatogen and subdermatogen. These two initials are ultimately responsible for the production of glumes and generative tissues, respectively. As NILSSON-LEISSNER (1925), ÅKERMAN (1927) and RÖSLER himself (*l. c.*) have already pointed out, this means that a chimera with glumes of speltoid appearance may very well have a progeny of normal plants all through, if the mutation has only occurred after the differentiation of the meristem into two initial groups.

Anatomical studies can hardly trace the problem of differentiation further back. At this point radiation experiments may be of great help. The present investigation permits the discussion to be brought only one step further, to the differentiation of the dormant primordium. The following data indicate, if this is composed of two or only one initial:

Year of experiment	X_1 material		N_1 material	
	1948	1949	1948	1949
Total number of speltoid chimeras	22	43	253	204
Ditto, with speltoids in the progeny, %	31,8	20,9	51,4	39,2
Total number of normal plants	793	356	870	325
Ditto, with speltoids in the progeny, %	12,5	3,7	16,1	11,7

As the speltoid chimeras are observed through the change of glume appearance and since the constitution of the germ line is checked by the progeny, the above given figures indicate a rather close correlation between dermatogen and subdermatogen occurrence of the speltoid mutation. This interrelation favours the idea that the primordia of the embryo are not clearly differentiated. It seems less acceptable to assume two initial groups to be already present in this stage and to explain the high correlation by frequent substitution. Under such a postulate, the

higher lethality in X_1 ought to be followed by a closer correlation between dermatogen and subdermatogen occurrence of the speltoid mutations, if compared with N_1. However, this is not the case, rather it is the other way around. Some observations indicate further that this undifferentiated initial of the dormant primordium must be composed of more than one cell. The fact that the correlation between dermatogen and subdermatogen registration of the speltoid mutation is not absolute, favours such an idea. The same holds true for the fact that the chimeras generally were chimerical within the single head. Another evidence that the head can hardly be the descendant of a single cell is given by a comparison between the normal : het speltoid ratio of the second against the following generations. In the 1948 experiment this ratio was 1 : 0,25 in the speltoid segregating X_1 and N_1 head progenies, while the ratio-type test later gave the average value 1 : 1,45. ANDERSON, LONGLEY, LI and RETHERFORD (1949) reached the same conclusion by studying the distribution of abnormal meioses in different parts of the tassel of maize irradiated in the dormant seed stage. They even calculated the number of cells in the initial to be seven or eight. From an analogous study on barley, CALDECOTT and SMITH (1952), however, reached the conclusion that all spore mother cells of a barley spike are the descendants of a single cell of the dormant embryo.

Even if it is possible anatomically to distinguish between different layers in the growing tip of the plant, these are hardly to be considered as absolutely separated. The ability to substitute is certainly considerable and this must become especially evident when the initial groups differ in vitality due to the induction of cytological disturbances. An indication of this is given by the progeny test of chimeras with an irregular pattern of normal and speltoid glumes. In the 1949 experiment, where such a classification in type was made, these irregular chimeras often bore the speltoid character in the germ line also (21,9 per cent). This low specialization of the different layers and thus their possibilities to substitute have also been observed by DERMEN (1947) from histogenetic studies on $2x$—$4x$-chimeras of cranberry.

Other indications of disturbed meristem in plants from irradiated seeds are the occurrence of heads with adventitious spikelets or branched tip (Fig. 2). The frequency of these non-heritable abnormalities are low but they may certainly be interpreted as secondary effects of the irradiation. The occurrence of supernumerary spikelets is an interesting phenocopy of a recessive heritable phenomenon which has appeared spontaneously (*cf.* COFFMAN, 1924; KAJANUS, 1924; NILSSON-LEISSNER,

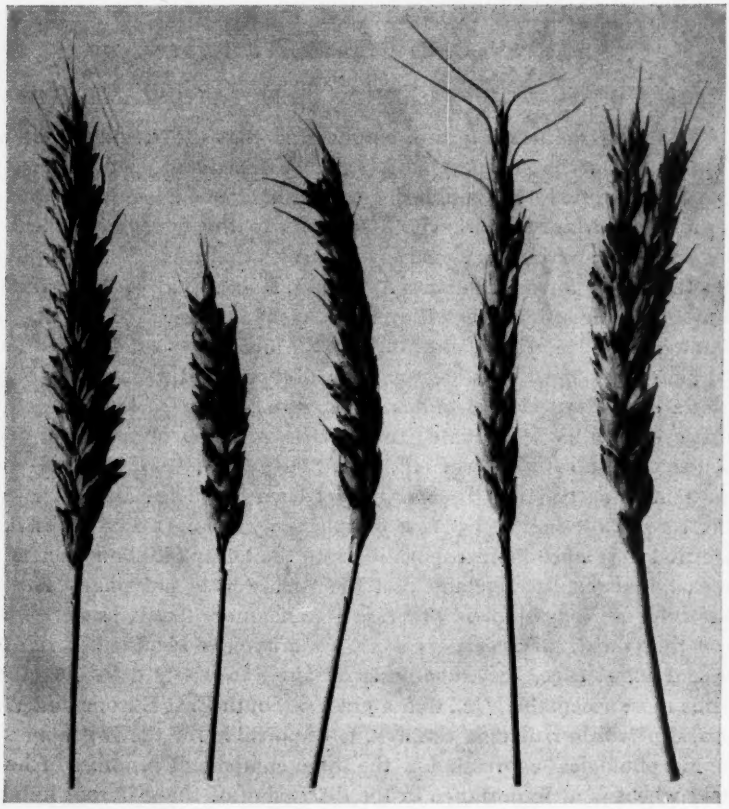


Fig. 2. Morphological ear abnormalities in the first generation from irradiated seeds of spring wheat (Rival). — From left to right: normal head, head with adventitious spikelets, speltoid chimera (lateral), speltoid head, and bifurcated head.

1925) and has in the present investigation been induced in connection with increased head density (*cf.* Fig. 82). The appearance of bifurcated ears is not uncommon in species crosses within *Triticum* (*cf.* NILSSON-LEISSNER, 1925; VAVILOV and JAKUSHKINA, 1925).

Besides these deformations of the X_1 and N_1 heads, a pronounced tendency to melanotic discolorations in the head region and the upper part of the straw should be mentioned as an effect of the irradiation. Even this character is observed in crosses and has been shown to be inherited by certain mutants (*cf.* pp. 146 and 151—152, Fig. 82).

IV. RATES OF MUTATIONS OBSERVED IN THE SECOND GENERATION.

1. THE SPONTANEOUS FREQUENCY OF MUTATIONS IN 6x-WHEAT.

In the introduction it was emphasized that the frequency of observable mutations in a polyploid is highly dependent on the degree of gene reduplication. The similarity of the component genomes thus is of great importance in the discussion. As to the hexaploid wheat, it now seems clear from very extensive analyses that it is an allopolyploid. The three component genomes A, B and D (also called A, B and C) cannot all be identical. According to independent results of KIHARA and LILIENFELD (1944, 1949) and MC FADDEN and SEARS (1944, 1946), the last added genome (D) is identical or closely related to that of a West-Asiatic diploid species, *Aegilops squarrosa* L. The basic genome (A) must certainly have originated from *Triticum monococcum* L. or some related, maybe extinct form (*cf.*, *e. g.*, SCHIEMANN, 1947; SEARS, 1948). The remaining genome (B) has not yet been identified and perhaps it may never be possible, because of the many changes that must have occurred in it since its incorporation into the tetraploid. Some scientists (*e. g.*, SCHRIMPF, 1951) claim that the first step to polyploidy has occurred by an autopolyploid process. It seems more likely, however, that even the two emmer genomes were not originally identical. From our present knowledge, the hypothesis of MC FADDEN and SEARS (1946) seems most acceptable, *i. e.*, that a grass of South-East Europe and West Asia, *Agropyron triticeum* GAERTN. is responsible for the B genome. As the morphological expression of the three component genomes of hexaploid wheat is of importance in the discussion of the different induced mutant types, the supposed parental species are shown in Fig. 3.

Even if the ancestry of the common wheat will never be cleared up in all its details, it is evident from the above that the species must have genes represented only once or twice. In some characters the hexaploid wheat will behave as a diploid in its genetic response to mutagens; in others the phenotypical registration of mutations observable in a diploid will be more or less completely buffered. The dissimilarity of the added genomes fits with the existence of easily observable spontaneous mutations. Their frequency could be expected to be comparatively high due to the increased chance of meiotic irregularities and the ability of the hexaploid to tolerate chromosomal alterations as a consequence of the partial reduplication of chromosome segments.

More accurate attempts to estimate the spontaneous frequency of

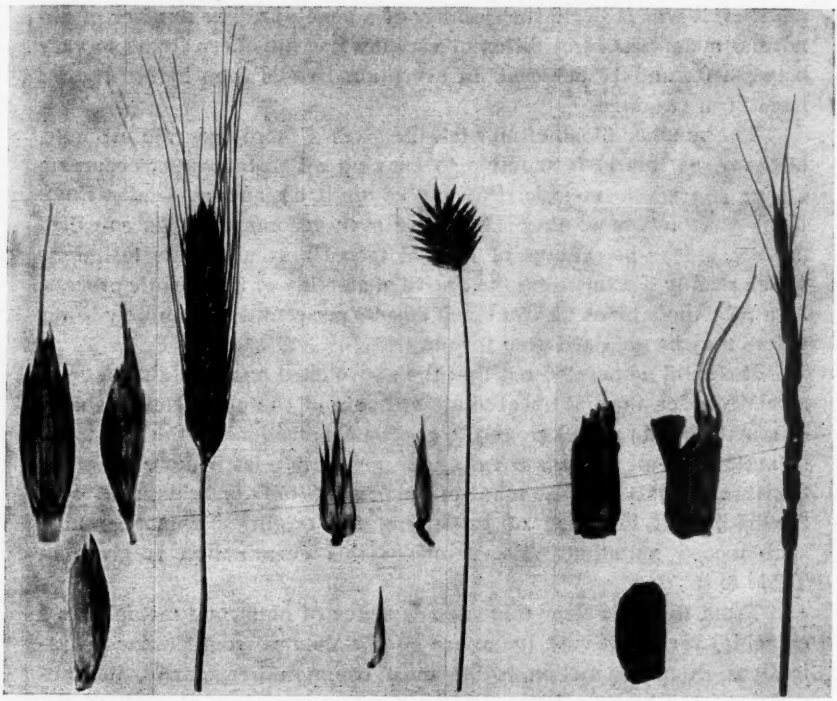


Fig. 3. The supposed parental species of the hexaploid wheat: *Triticum monococcum* ($2n=14$, genome A), *Agropyron triticeum* ($2n=14$, genome B) and *Aegilops squarrosa* ($2n=14$, genome D).

mutations in wheat never seem to have been made, not even as a basis for comparison in mutation experiments. Quite exact analyses — but from this point of view incomplete — are, however, made on a cytological basis. Estimates of meiotic disturbances as a measure of the genetic stability of wheat varieties have been used by SAPEHIN (1928), THOMPSON and ROBERTSON (1930), HOLLINGSHEAD (1932), POWERS (1932 a, b and c), MORITZ-VOM BERG (1935), MYERS and POWERS (1938), LOVE (1940 b, 1951), and CAMARA (1945). UCHIKAWA's (1941) analyses on normal plants descending from het speltoids may also illustrate the same question. In most cases only the metaphase I of PMC has been studied and the frequency of univalents alone or together with polyvalents has been used as a measure of genetic stability. Even if such an analysis will not be able to grasp the total frequency of mutated

gametes, it will indicate the stability of a genotype. The number of abnormal metaphases I in different varieties has thus been found to vary between 0,6 and 10 per cent. In exceptional cases even higher figures have been obtained.

The number of abnormal tetrads gives a more accurate estimate, however, as this is better able to sum up all disturbances occurring during the whole meiosis. POWERS *et al.* (*l. c.*) and especially LOVE (*l. c.*) were aware of this. The latter even recommends his so-called meiotic index (percentage of normal tetrads) as a routine technique which readily demonstrates the degree of stability of the meiotic process. As a rule, these index figures reach 95—99 per cent, but definitely lower values may be obtained even for pure strains.

It should be pointed out that the above cited analyses aim more at a relative idea than at an absolute estimate of the cytological stability of a material. As a background to genetic observations of the frequency of mutations, an analysis covering all meiotic phases is more accurate. Such an analysis was also made in the present study of Scandia III with the additional intention of explaining the origin of some common spontaneous mutations. The result of this examination is given in Table 3.

From this it is clear that the frequency of abnormal metaphases I coincides very well with the above cited estimates from similar investigations. Non-conjunction is the most common irregularity, its consequence can be traced through the whole meiosis and it explains the rare production of monosomic plants. If the low number of observations in late anaphase I should be representative, nearly half of the univalents will, however, reach the poles in due time. The higher frequency of univalents in telophase I may be a more accurate evaluation but is logical when considering the evidence of non-oriented bivalents. The frequency of these abnormalities has been discreetly omitted in Table 3, as such a count must be less reliable on smear preparations. The phenomenon can, however, not be classified entirely as an artefact, since it was observed by POWERS (1932 a, b and c) in paraffin-embedded and sectioned material. According to his observations, it seems highly probable that by dividing late, non-oriented bivalents may give rise to lagging chromosomes and finally to micronuclei.

In rare cases the two homologous univalents go to the same pole which may give $(n+1)$-gametes and the possibility of trisomic offspring. Such a production of $(n+1)$-gametes may also arise through non-disjunction in the homotypic division. Another source of aneu-

TABLE 3. *Analysis of meiosis in control plants of Scandia III (Tr. vulgare).*

I. First meiotic division

TABLE 3. *Analysis of meiosis in control plants of Scandia III (Tr. vulgare).*

I. First meiotic division.

Plant No.	Metaphase I:						Late anaphase I:					Telophase I:						Interkinesis:									
	2_{II}	$2_{II}+2_{I}$	$1_{II}+4_{I}$	$1_{II}+1_{III}+1_{I}$	$1_{II}+1_{IV}$	abnormal, % of total	0_{I}	1_{I}	2_{I}	fragment	abnormal, % of total	0_{I}	1_{I}	2_{I}	misdivision	fragment	abnormal, % of total	0+0	0+1	0+2	1+1	1+2	0+fragm.	0+1 & fragm.	1+fragm.	1+1 & fragm.	abnormal, % of total
1950—1	247	4	—	—	1	2,0	1	—	—	—	—	4	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—
» —2	309	8	1	—	2	3,4	35	—	1	1	5,4	45	2	1	—	—	6,3	284	5	—	—	1	1	—	—	—	2,4
» —3	616	22	2	2	3	4,5	111	—	3	—	2,6	155	2	4	1	5	7,2	427	8	—	—	—	4	1	1	—	3,2
» —4	77	3	—	—	—	3,8	17	—	1	—	5,6	49	1	—	1	3	9,3	152	2	—	—	—	1	—	—	—	1,9
1951—1	398	12	1	1	4	4,3	19	—	—	1	5,0	256	3	—	—	7	3,8	301	3	—	2	—	1	—	4	1	3,5
» —2	342	14	—	—	1	4,2	71	1	—	—	1,4	173	6	1	—	4	6,0	204	2	—	—	—	4	—	—	—	2,9
» —3	211	3	1	1	6	5,0	16	—	—	—	0,0	73	3	2	—	1	7,6	122	2	1	1	1	—	—	1	1	5,4
» —4	326	9	—	1	8	5,2	8	—	—	—	—	251	8	3	1	2	5,3	107	3	—	—	—	1	—	1	—	4,5
Total	2526	75	5	5	25	4,2	278	1	5	2	2,8	1006	25	11	3	22	5,7	1598	25	1	3	2	12	1	7	2	3,2

II. Second meiotic division.

Plant No.	Metaphase II:				Late anaphase II:			Telophase II:					Tetrads:										
	0+0	0+1	1+1	abnormal, % of total	0+0	0+1	abnormal, % of total	0+0	0+1	1+1	0+misdivision	abnormal, % of total	0+0+0+0	0+0+0+1	0+0+0+2	0+0+1+1	0+0+1+2	0+1+1+3	0+0+0+ +fragm.	0+0+1+ +fragm.	0+1+1+ +fragm.	0+2+misdiv.+ +fragm.	abnormal, % of total
1950—1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
» —2	49	1	—	2,0	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
» —3	21	—	—	0,0	13	—	0,0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
» —4	151	6	1	4,4	25	1	3,8	91	3	1	—	4,2	255	—	—	—	—	—	1	1	1	—	1,2
1951—1	88	2	1	3,3	10	1	9,1	59	5	—	—	7,8	478	8	2	2	1	1	3	1	—	—	3,6
» —2	40	3	—	7,0	7	—	—	3	—	—	—	—	171	2	—	—	—	—	—	—	—	—	1,2
» —3	76	4	—	5,0	8	—	—	95	—	1	1	2,1	398	7	—	1	—	—	1	—	—	—	2,2
» —4	39	3	—	7,1	25	—	0,0	43	—	—	—	0,0	601	13	1	6	—	—	1	1	—	1	3,7
Total	464	19	2	4,3	89	2	2,2	291	8	2	1	3,6	1903	30	3	9	1	1	6	3	1	1	2,8

ploidy is the occurrence of polyvalents which may also cause less drastic rearrangements.

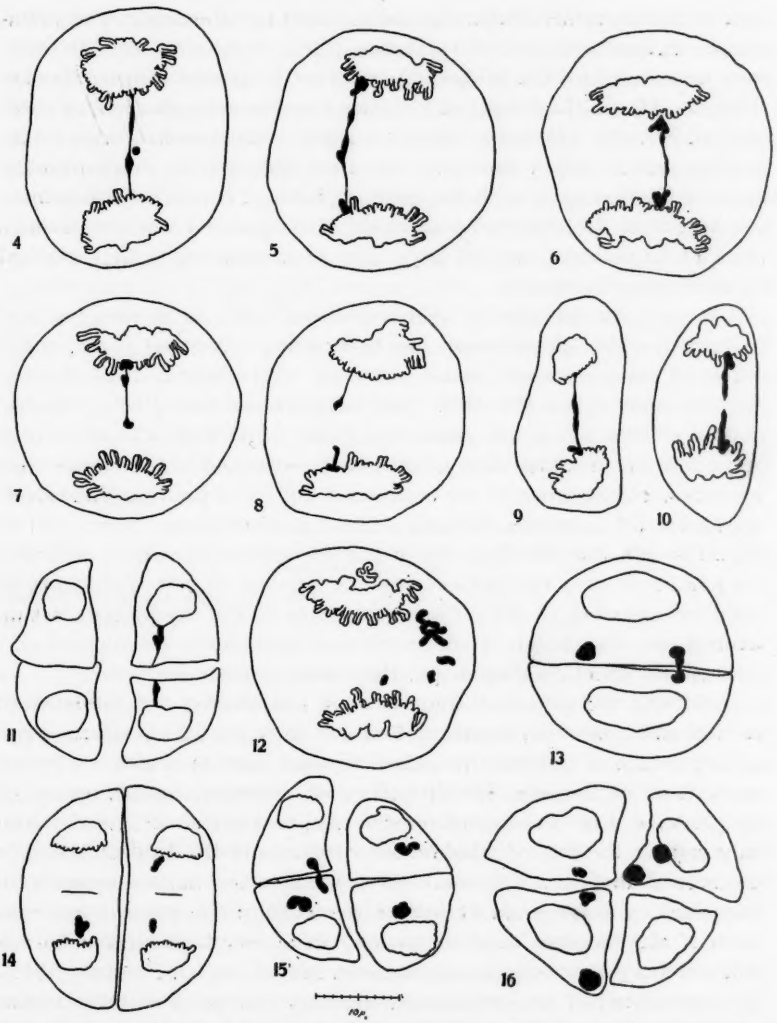
Besides the evidence for loss or addition of whole chromosomes and chances of non-homologous crossing-over, the cytological analysis of normal plants indicates that deficiencies in several cases may be the intrinsic cause of off-types. The mechanism behind these deficiencies is not always the same. At least three different types of fragmentation have been observed. In two cases the process of deletion is associated with the formation of bridges, the frequencies of which are the following:

	Late A I	T I	Late A II	T II
No. of PMC's without bridge	261	1057	89	300
No. of PMC's with bridge	25	10	2	2
Percentage of PMC with bridge	8,7	0,9	2,2	0,7

One type of bridge is associated with a small fragment (Fig. 4) and is extremely rare. Only two such cases were observed in the present analysis and both in telophase I. Similar configurations were earlier found in interspecific crosses of *Triticinae* (*cf.* AASE, 1946), in *Tradescantiae* (DARLINGTON, 1937) and in *Secale* (MÜNTZING and PRAKKEN, 1941), where they were interpreted as the result of inversion crossing-over.

The second type of bridge is not associated with any visible fragment (Figs. 5 and 6). Only when these bridges are broken (Figs. 7 and 8) will there be a chance for deletions to occur. Similar bridges were earlier observed in *Triticum Spelta* (SANCHEZ-MONGE, 1949), as well as in *Tradescantiae* (DARLINGTON, 1937; DARLINGTON and UPCOTT, 1941), in asynaptic *Pisum* (KOLLER, 1938), in haploid *Secale* (LEVAN, 1942), and in interspecific crosses of *Bromus* (WALTERS, 1950, 1952). Even POWERS (1932 a) must have met the same phenomenon in his wheat material, since he illustrated fragmentation with two pictures, among others (his Figs. 2: H and 4: B), of exactly the same appearance as that of the present Fig. 8. He only touches on this type of fragmentation in the text, however, and does not mention the existence of bridges at all.

These bridges without fragments were generally considered the result of sister reunion or terminal non-division of broken and of unbroken chromatids. In the heterotypic division, sister reunion can hardly be the explanation of the bridges found in the present study. Neither the appearance of the bridges with a marked non-disjunctive



Figs. 4—16. Cytological abnormalities in the meiosis of untreated wheat (Scandia III) resulting in fragmentation. — Fig. 4, T I bridge with fragment. — Figs. 5—8, delayed terminalization at T I with risk of fragmentation in the event of a breakage. — Figs. 9—11, bridge formation at T II and in the tetrad. — Fig. 12, a fragment and two univalents at T I, of which one chromatid is a-misdivided. — Fig. 13, two univalents at interkinesis, one exposed to p-misdivision. — Fig. 14, a-misdivision at T II. — Figs. 15—16, tetrad with four daughter univalents, of which one about to break and the other broken by p-misdivision.

section in the middle of the chromatid string nor the analysis of metaphase I favour such an interpretation. This explanation cannot, however, be refused for the bridges observed in the second meiotic division (Figs. 9—11), as the breaks of the first division may give rise to sister reunion (*cf.* MC CLINTOCK, 1941). A further discussion of this phenomenon will be taken up later, since new light will be thrown on the question in connection with the speltoid problem. It will only be pointed out here that the observed fragments at telophase I and interkinesis (2,1 and 1,3 per cent, respectively) must to a high degree be explained by this bridge formation.

A very special type of fragmentation is the misdivision of the centromere. This phenomenon has been observed several times on the univalent of monosomic plants (WINGE, 1924; SEARS, 1946, 1952 a; SANCHEZ-MONGE and MAC KEY, 1948; LI, HSIA and LEE, 1948; SANCHEZ-MONGE, 1950 a and b). As shown by Table 3 and Figs. 12—16, it may occur in a meiosis including all 42 chromosomes as well but only as a secondary consequence of the occasional failure of pairing. The releasing forces (*cf.* SANCHEZ-MONGE's a- and p-misdivision, 1950 a and b; Figs. 12—16), the time these set in and the disruption pattern will vary the end result of a misdivision. Besides unequal chances for incorporation or exclusion of the different products of the misdivision, a subsequent non-disjunction of telocentric chromosomes or chromatids may increase the genetic variation (*cf.* DARLINGTON, 1940).

Already the observed frequency of aneuploidy, non-homologous pairing, disorientation, fragmentation and their secondary effects, gives a higher rate of meiotic irregularities than can be registered by an analysis of the tetrads. The frequency of abnormal tetrads averaging 2,8 per cent and corresponding well with earlier cited investigations only reflects the loss of whole chromosomes and the fragmentation in the second division. The total cytologically observable frequency of irregularities must reach at least 4 per cent in the present material. Even if chromosome aberrations may to a very high degree be responsible for the observable spontaneous mutations (*cf.* HUSKINS, 1941), the possibilities of invisible rearrangements and point mutations must also be considered. The spontaneous frequency of mutations is further the sum of transmitted irregularities from the microspore and the megaspore. To this must be added somatic mutations in the germ line. Cytologically, then, the *a priori* condition for the production of off-types in the progeny of an inbred wheat homozygote is considerable.

In order to check that by a genetic analysis and also to furnish the

necessary background to the induced frequency of mutations, the spontaneous rate of mutations has carefully been determined in the two varieties used, Scandia III (winter wheat) and Rival (spring wheat). The uncertainty of such an analysis must not be neglected, however. A crossbred wheat variety seldom is a real pure line. Outcrossing may always occur (*cf.* NILSSON-EHLE, 1915; JENKIN, 1925). A repeated individual selection of the variety before use, a comparatively cold and moist climate to prevent open-flowering, a test field as well isolated as possible and a direct analysis of a space-planted stand to avoid intermixture are the conditions to ensure safety. A further strengthening of the analysis is obtained by consistently, generation after generation, basing it on separated plant progenies. In so doing, intermixtures are more easily detectable and the off-types are easier to classify as true mutations or products of outcrossing. With these precautionary measures taken, and with the analysis running for a period of years, the control material of Scandia III and Rival showed the following phenotypic irregularities:

	No. of analysed plant progenies	No. of plants in the progeny test	No. of events of mutation		No. of events of outcrossing	
			abs.	%	abs.	%
Scandia III, winter wheat	686	33.458	13	1,9	3	0,4
Rival, spring wheat	605	29.470	8	1,3	1	0,2

The carefully tested mutations were 4 speltoid, 8 lax-eared and 1 bearded type in Scandia III; 4 speltoid, 2 lax-eared and 2 short-strawed types in Rival. All speltoids except one were monosomic. The bearded mutation does not show any cytological or genetical irregularities and is thus probably a point mutation. The lax-eared mutations were heterogeneous as to origin and showed completely normal to definitely disturbed meiotic balance. The short-strawed mutations were caused by chromosome aberrations. The events of outcrossing could clearly be separated, as they included several independent segregating off-characters, at least one of dominant behaviour for each case.

No other analysis of the spontaneous frequency of mutations in wheat seems to have been made with similar accuracy and on a material large enough to give a more reliable comparison. In earlier X-ray experiments, where controls were reported, these were too modest to allow any statement. In no case, however, has a spontaneous mutation been reported. In experiments on the mutagenic effect of temperature and storing, more extensive controls were presented. Here, however, the account was less satisfactorily handled, since the rates are given as

the number of phenotypically deviating plants in a bulk plot. No information was thus available about the number of individuals descending from one and the same event of mutation and checks are failing as to whether the off-types were mere modifications. Summing up the results from experiments of that kind, DELAUNAY (1934) estimated the spontaneous frequency of mutants in wheat to be 0,05—0,1 per cent. This value, depending somewhat on the variety tested, is only slightly higher than those obtained in the present study, if the data here were calculated in the same way.

The high ability of wheat to phenotypical buffering may cause even aneuploid plants to be indistinguishable from type (*cf.* SEARS, 1944). A lower frequency of observable mutations than that expected from cytological irregularities is thus quite natural. The process of fertilization and the first stages of zygotic development mean an additional important sifting mechanism. The precision of the delicate mechanism of cell division is easily overestimated by mere genetic observations.

Before leaving the question of spontaneous mutability of wheat, it should be pointed out, that the analysis reported above only holds for stable varieties. Hybridization and especially crosses between widely separated varieties considerably increase the frequency of irregularities. The possibilities of pure mutations besides the ordinary recombinations may be very high (*cf.* p. 100).

2. THE FREQUENCY OF INDUCED MUTATIONS IN 6x-WHEAT.

In spite of the fact that many experiments have been made on induced mutations in polyploids, there are exremely few reports on the total frequency obtained with different doses. No such investigation has been presented, where the hereditability of the observed off-types has been verified by a progeny test.

To carry out a more thorough study is, however, not only tedious and precarious work but demands great accuracy. Even if one has chosen to treat the dormant seed as being the most reproducible method, and has applied exact doses and works with an extremely uniform strain, an attempt to determine the total frequency of observable mutations may give rather varying results. An intervening factor has already been discussed, *viz.* the counter-selection caused by the physiological toxicity of the treatment. This effect is well illustrated by Tables 1 and 4. The lethality of X_1 is greater in the 1949 than in the 1948 experiments and as a consequence the frequency of mutations is reduced in the surviving X_1 plants. Similarly, Table 4 indicates that the lower

TABLE 4. *Rates of mutation in the second generation of X-ray and neutron treated 6x-wheat.*

Mutation rate

TABLE 4. *Rates of mutation in the second generation of X-ray and neutron treated 6x-wheat.*

Year of treatment	Dose	No. of plant progenies examined	In the 2nd generation observed frequency of:														Total No. of mutations	Mutation rate per	
			Speltoid mutations		Dense-eared mutations		Lax-eared mutations		Bearded mutations		Short-strawed mutations		Chloro-phyll mutations		Other mutations			plant progeny, %	plant progeny & dose unit, $\cdot 10^{-4}$
			abs.	%	abs.	%	abs.	%	abs.	%	abs.	%	abs.	%	abs.	%			
										Winter wheat (Scandia III):									
1944	Control	311	—	0,0	—	0,0	1	0,3	—	0,0	—	0,0	—	0,0	—	0,0	1	0,3	—
»	20.000 *r*	426	66	15,5	43	10,1	151	35,4	4	0,9	60	14,1	1	0,2	2	0,5	327	76,8	0,4
										Spring wheat (Rival):									
1948	Control	275	—	0,0	1	0,4	1	0,4	—	0,0	—	0,0	—	0,0	—	0,0	2	0,7	—
1949	»	200	—	0,0	—	0,0	1	0,5	—	0,0	—	0,0	—	0,0	—	0,0	1	0,5	—
1948	5.000 *r*	200	11	5,5	7	3,5	18	9,0	1	0,5	3	1,5	1	0,5	2	1,0	43	21,5	0,4
»	10.000 »	200	34	17,0	20	10,0	26	13,0	1	0,5	11	5,5	1	0,5	2	1,0	95	47,5	0,5
»	15.000 »	185	29	15,7	26	14,1	27	14,6	2	1,1	12	6,5	—	0,0	2	1,1	98	53,0	0,4
»	20.000 »	133	21	15,8	16	12,0	16	12,0	1	0,8	7	5,3	—	0,0	2	1,5	63	47,4	0,2
»	25.000 »	97	11	11,3	8	8,2	10	10,3	1	1,0	5	5,2	—	0,0	—	0,0	35	36,1	0,1
1949	5.000 »	100	9	9,0	12	12,0	11	11,0	—	0,0	5	5,0	—	0,0	1	1,0	38	38,0	0,8
»	10.000 »	55	3	5,5	4	7,3	8	14,5	—	0,0	2	3,6	—	0,0	—	0,0	17	30,9	0,3
»	15.000 »	85	5	5,9	6	7,1	9	10,6	—	0,0	1	1,2	—	0,0	—	0,0	21	24,7	0,2
»	20.000 »	72	5	6,9	6	8,3	7	9,7	—	0,0	2	2,8	—	0,0	—	0,0	20	27,8	0,1
»	25.000 »	80	4	5,0	6	7,5	8	10,0	—	0,0	3	3,8	—	0,0	1	1,3	22	27,5	0,1
1948	2.100 *dis*	200	22	11,0	16	8,0	25	12,5	—	0,0	5	2,5	—	0,0	2	1,0	70	35,0	1,7
»	3.200 »	200	36	18,0	20	10,0	28	14,0	2	1,0	8	4,0	—	0,0	5	2,5	99	49,5	1,5
»	3.300 »	200	47	23,5	33	16,5	49	24,5	2	1,0	17	8,5	—	0,0	2	1,0	150	75,0	2,3
»	5.700 »	200	67	33,5	38	19,0	54	27,0	2	1,0	37	18,5	—	0,0	—	0,0	198	99,0	1,7
»	8.600 »	200	78	39,0	45	22,5	77	38,5	3	1,5	43	21,5	—	0,0	2	1,0	248	124,0	1,4
1949	2.900 »	100	13	13,0	6	6,0	11	11,0	1	1,0	3	3,0	—	0,0	—	0,0	34	34,0	1,2
»	5.800 »	100	16	16,0	20	20,0	32	32,0	2	2,0	5	5,0	—	0,0	—	0,0	75	75,0	1,3
»	10.600 »	100	35	35,0	28	28,0	45	45,0	3	3,0	16	16,0	—	0,0	2	2,0	129	129,0	1,2
»	16.800 »	100	39	39,0	23	23,0	70	70,0	1	1,0	30	30,0	1	1,0	3	3,0	167	167,0	1,0

physiological radiosensitivity of the winter wheat results in a higher frequency of mutations wilh equal dose than in the spring wheat. The interannual variation is not observable after neutron treatment, since this is characterized more by a purely genetic action and is thus less effected by environmental conditions. For practical mutation breeding, these observations mean that the chances for seeds of low vitality or with unfavourable conditions for germination to give good results may be considerably limited. When the death of the seedling is not genetically conditioned, it is not to be expected that only negative mutations will be lost.

A second factor, also of great practical interest, is the intervarietal difference in the genetic and phenotypic stability against mutagenic treatments. This problem has earlier been discussed by GUSTAFSSON (1947). He showed that the old Golden barley, a line-selection from an indigenous variety, is less inclined to mutate than the decidedly younger Maja and Ymer barley, both produced by cross breeding. It is also the opinion of the present writer that an incomplete genetic balance increases the tendency to mutate through external influences. A high frequency of spontaneous mutations is generally followed by an increased ability to mutate after mutagenic treatments. If the highest possible number of mutations is desirable from a practical point of view, cross material of an early F-generation is to be recommended.

Without considering the above-mentioned factors influencing the reproducibility, some apprehensions remain if one attempts to measure the total frequency of observable mutations. Observable is an indistinct term. In the present study it means a clearly registrable departure from type. This definitely implies an undervaluation of the frequency of mutations, since all induced modifiers must be neglected. In wheat, it is also hardly possible phenotypically to identify the chromosome mutations, where decreased fertility is the only phenotypic effect. The high buffering ability will here cause too small differences, often overshadowed by attacks of wheat midge, *etc.* This type of mutation was also omitted in the present considerations because of its little value for the main aspects that were set up for these rate analyses.

The claim to a registrable departure from mother type means that every estimation of the frequency of mutations is to some extent relative. Mutations to ear density will, for example, be more easily expressed phenotypically in a lax-eared than in a dense-eared mother type. This varietal limitation of phenotypical expression is not to be neglected.

In spite of this inherent imperfection of all attempts to determine

the total frequency of observable mutations, such an analysis has been performed on the present material. The aim of the study has been to analyse the mutagenic reaction of a diploid and a polyploid from the aspects of evolution and plant breeding. By this relative mode of attacking the problem and with the experiments parallelly treated according to the same principles, the unreliability of the test should not be so great that the obtained differences in the mode of reaction may not be considered as highly representative.

The frequency of observed mutations in X_2 and N_2 of the wheat experiments is given in Table 4. Before discussing the rates given here per plant progeny, since this is to be preferred to per head progeny (*cf.* MAC KEY, 1951), some comments should be given about their accuracy. As pointed out earlier, the X_2 material was sown as plant progenies in the winter wheat, while each X_1 and N_1 plant was tested through at the most three head progenies in the spring wheat. All off-type individuals in X_2 and N_2 were picked out, and in the winter wheat material two normal plants of each X_1 progeny as well. In spite of the fact that the total progeny test of the selected plants comprised 3.100 progeny plots or 118.682 individual daughter plants in the winter wheat, the frequency values of this experiment given in Table 4 are not completely free from objections. A considerable part of the X_3 material totally outwintered in 1947. A certain reconstruction was possible by the aid of preserved head samples but nearly one third of the mutations thus missed a definite classification. The progeny test is, however, complete for the 1948 experiment with spring wheat. This test comprised 2.254 progeny plots or about 119.000 daughter plants. For financial reasons, the 1949 experiment was only progeny-tested for mutations of practical interest. The good interannual conformity of the neutron experiments, where environmental influences are at a minimum, and the experience of the progeny test of the 1948 trial, indicate that the miscalculation of the mutation frequency of the 1949 experiment cannot be considerable. A certain overestimation by including mere modifications is bound to occur but it seems fairly well counterbalanced by the progeny test which separates phenotypically similar but genotypically dissimilar mutants of one and the same pedigree.

In order to give a background to the rate values obtained on 6*x*-wheat, these have been presented in diagrammatic form in Fig. 17 together with analogical data from the 1947, 1948 and 1949 experiments on 2*x*-barley. The following conclusions can be made: (i) The more purely genetically operating neutrons no doubt reveal a higher, pheno-

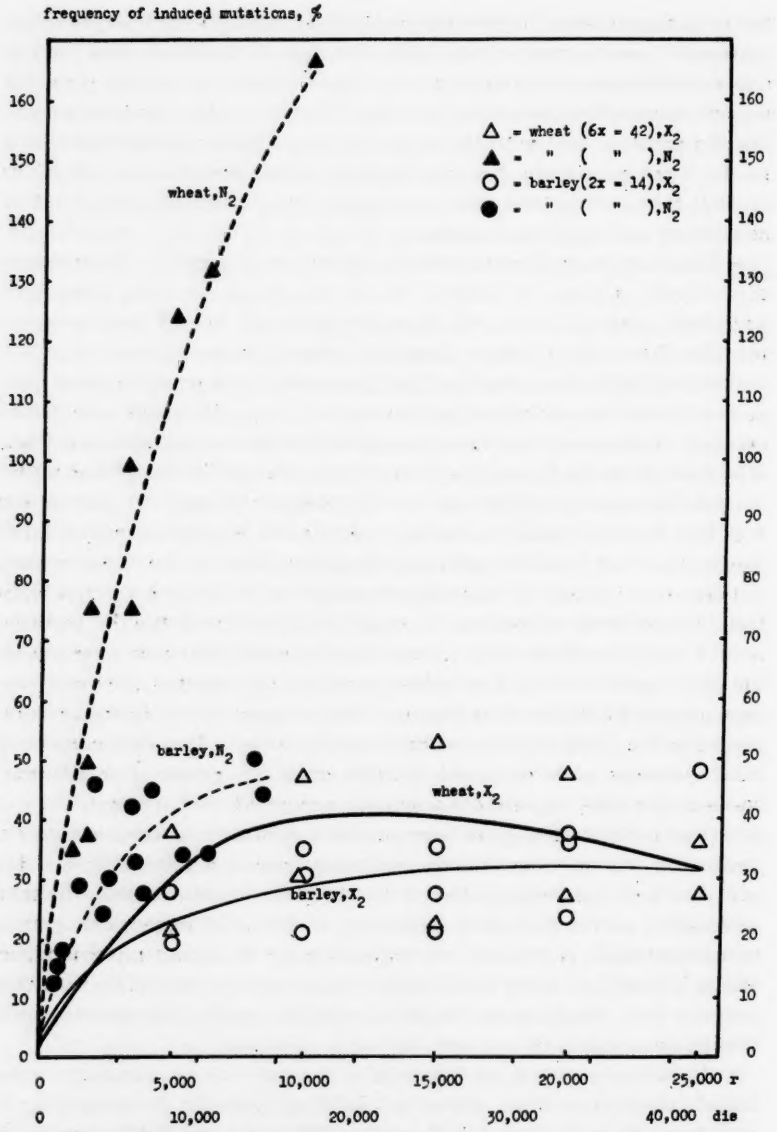


Fig. 17. The total frequency of observable mutations in the second generation of 6*x*-wheat (Rival) and 2*x*-barley (Bonus) after irradiating dry seeds with successively increasing doses of X-rays and fast neutrons. Inter-radiational dose scale: 1 *dis*=0,6 *r*.

typically identifiable mutation capacity in the allohexaploid than in the diploid. — (ii) X-rays have not the same possibilities as neutrons to reveal differences in mutability from species to species. The reason is the non-genetic counter-selection due to the high physiological toxicity (see above). — (iii) The higher genetic effect of fast neutrons when compared with X-rays must mean that the main part of the observed mutations in the polyploid as well as in the diploid are chromosome aberrations (*cf.* p. 71). — (iv) Due to the lower tolerance to chromosomal alterations the $2x$-barley reaches its maximum mutation frequency sooner than the $6x$-wheat with successively increasing doses. This behaviour is overshadowed by the non-genetic effects of X-rays but is easily discerned in the neutron experiments.

The pronounced differences in the mode of reaction of a diploid and a polyploid species and the fact that the applied doses have been chosen rather high for practical reasons, hamper and impair the value of detailed statistical analyses. Some data should be added, however, to enlarge upon and verify the statements made from Table 4 and Fig. 17. Thus, the assertion of the registered mutations being chromosome aberrations can be verified by checking the fertility of mutated against non-mutated X_1 and N_1 spikes. For barley a strong correlation has already been found between frequency of chlorophyll mutations and fertility in X_1 by GUSTAFSSON (1940), FREISLEBEN and LEIN (1943) and in X_1 and N_1 by MAC KEY (1951). As for wheat, an even closer association should be expected between the phenotypically identifiable mutations and changes in chromosome structure. On the other hand, this relation should be more faintly registered by the fertility due to the high tolerance of the polyploid to cytological disturbances.

In the neutron experiments this interrelation is, however, easily discerned also for $6x$-wheat as evident from the χ^2-analysis below on the 1949 experiment (that of 1948 was omitted as less reliable on this special point due to the attack of wheat midge). The test is based on head and not plant fertility, since this considerably sharpens the analysis:

	Fertility of N_1 heads:							
	0 — 70	— 75	— 80	— 85	— 90	— 95	— 100 %	
No. of non-mutated N_1 heads ..	146	73	89	99	93	51	18	=569
Expected	187,02	74,81	77,64	92,38	75,94	45,34	15,87	
No. of mutated N_1 heads	184	59	48	64	41	29	10	=435
Expected	142,98	57,19	59,36	70,62	58,06	34,66	12,13	

$\chi^2 = 36{,}93$; d. f. $= 6$; $P < 0{,}001$.

In the X-ray experiments, this accumulation of mutations in heads of low fertility is hardly to be found:

	Fertility of X_1 heads:							
	0 — 70	— 75	— 80	— 85	— 90	— 95	— 100 %	
No. of non-mutated X_1 heads	57	63	88	131	250	233	129	=951
Expected	59,55	61,35	93,84	129,93	240,91	231,88	133,54	
No. of mutated X_1 heads ...	9	5	16	13	17	24	19	=103
Expected	6,45	6,65	10,16	14,07	26,09	25,12	14,46	

$\chi^2=10{,}52$; d. f.=6; P=0,11.

For the same X-ray doses as those applied in the present study, FRÖIER, GELIN and GUSTAFSSON (1941) have reported a frequency of disturbed cells of the first mitotic cycle of the irradiated embryo varying from 29,4 per cent (5.000 *r*) up to 95,8 per cent (25.000 *r*). With the neutron doses used here, the comparable figures must be definitely lower, if NYBOM's earlier cited interrelationship $dis/r \simeq 0{,}6$ can be accepted not only for barley but also for wheat. Thus, the inability of X_1 in contrast to N_1 to show an accumulation of mutations to heads of low fertility, *i. e.*, heads including gross chromosomal alterations, again offers good evidence of the »screening mechanism» which the non-genetic lethality of X-rays actually is. The neutron experiments prove that the somatic cell has a much higher resistance to genetic irregularities than was earlier supposed from mere experiments with X-rays. The elimination of chromosome aberrations is more a physiological than a genetic process, *i. e.*, the removal is due to indirect effects of the radiation connected with damage of the plasm constituents. This view on the intrasomatic selection presented by me already in 1951 has recently been verified by my colleagues (EHRENBERG *et al.* 1953).

The pronounced accumulation in N_1 of mutations to heads of low fertility indicates that the mutations are here induced by more severe chromosomal disturbances than in X_1. An analysis of heterogeneity on the above presented data on mutated N_1 and X_1 heads confirms this ($\chi^2=100{,}68$; d. f.=6; $P<0{,}001$). FANO (1944) and MAC KEY (1951) reached the same conclusion when studying recessive lethals of *Drosophila* and chlorophyll mutations of barley, respectively. It is thus reasonable to expect a higher frequency of drastic mutations in N_2 than in X_2. It is, however, very questionable if this difference could be used as evidence that true gene mutations of phenotypical penetration should be more frequent with X-ray than with fast neutrons. Everything goes

to prove that the overwhelming majority of X-ray induced observable mutations in 6*x*-wheat must be caused by chromosomal rearrangements.

That there should be more a quantitative than qualitative difference between observable mutations in wheat induced by X-rays and neutrons is supported by the good conformity of the two mutagens in their ability to produce different types of mutations. An exact proof of such a conformity can only be made by a genotypic grouping of the different mutations. For financial reasons such an analysis can hardly be made on the total material, however. A test based on the phenotypic grouping used in Table 4 may suffice. As a detailed analysis has not revealed any difference in the relative frequency of the different types of mutations as to dose, only the test of heterogeneity between years and mutagens will be presented here:

	χ^2	d. f.	P-value
1948 experiment, X_2 *versus* N_2	6,51	5[1]	0,26
1949 » » » »	5,84	4[2]	0,26
X_2, between years	7,64	4[2]	0,15
N_2, » »	11,31	5[1]	0,05

[1] Chlorophyll-defective and »other» mutations bulked into one group.
[2] Bearded, chlorophyll-defective and »other» mutations bulked into one group.

This phenotypical conformity of the direction of the mutation process between X-rays and neutrons is supported by a similar genotypic analysis of the induced speltoids (*cf.* p. 128). The slight tendency to an interannual difference may be explained by one experiment being progeny-tested, the other not. In the latter case, both X_2 and N_2 show a surplus of lax-eared mutations but a deficit of speltoids. A progeny test often reveals the lax-eared off-type to be a mere modification, while different types of recombination from a single event seem especially common to speltoids.

Another problem of great interest is how the higher frequency of observable mutations in a polyploid as compared with a diploid is to be explained. As stated in the introduction, at least two explanations may be given. The higher absolute frequency of chromosome aberrations due to the increased number of chromosomes may explain the result, if the degree of gene reduplication is not too high. On the other hand, the polyploid has a greater tolerance to genetic disturbances, which will better enable it to utilize all possible recombinations from every single chromosomal rearrangement.

If the last explanation is valid, a non-random distribution of mut-

ations should be expected in the 6x-wheat. In the progenies of the X_1 and N_1 heads a tendency to include two or more different mutations should thus exceed a randomized Poisson-distribution. That this is the case can be shown by a probability analysis, where the whole spring wheat material has been treated together. The bulking is justified by the absence of any variation between doses, mutagens or years, a conformity which is expected in the case of a common origin of the associated mutations:

	No. of head progenies with a mutation number of: 0	1	2	>2	
1948—49, X- and N-material	5185	1054	334	50	=6623
Expected	4991,76	1411,36	199,35	20,53	
Difference	+193	—357	+135	+29	

χ^2=231,21; d. f.=3; P < 0,001.

It should be pointed out that this grouping of mutations may be given another explanation. If an ionizing particle has a range long enough to produce more than one hit, such an accumulation should be expected. NAGAI and LOCHER (1938) and NISHINA and MORIWAKI (1939, 1941) have accepted this interpretation in their studies on sex-linked lethals of *Drosophila*, where neutrons in contrast to X-rays were reported to cause a grouping of the induced mutations. FANO (1943, 1944) has, however, made a reexamination of their findings and disclaims such a non-random distribution of mutations induced by neutrons. MAC KEY (1951) has reached the same conclusion from his study of chlorophyll mutations in barley. The same result is obtained if all observable mutations are considered. Thus, the pronounced grouping effect observed in the 6x-wheat for both X-rays and neutrons must be due to the chromosomal constitution of the species, and not to the mutagenic treatment. Since this tendency to accumulation does not exist in 2x-barley, it must further be considered a characteristic of polyploidy. In sharp contrast to the 6x-wheat, the 2x-barley shows a good random distribution of induced mutations. There is even a slight deficit of spikes with two or more mutations, which further confirms the low tolerance to chromosome disturbances in a diploid:

	No. of spike progenies with a mutation number of: 0	1	2	>2	
1947—49, X- and N-material	3599	602	39	1	=4241
Expected	3610,15	581,40	46,82	2,63	
Difference	—11	+21	—8	—2	

χ^2=3,08; d. f.=3; P=0,39.

The simultaneous induction of more than one type of mutation in 6*x*-wheat can also be illustrated by numerous detailed analyses (see below). There is no doubt that the higher frequency of observable mutations of the 6*x*-wheat is due more to the better tolerance to chromosome disturbances and the high recombining ability as a consequence of this, than to the mere increase in the number of genes.

V. ANALYSIS OF THE INDUCED MUTATIONS.

The pronounced grouping of the induced mutations indicates that the »diploid sector» of the germ plasm of 6*x*-wheat is restricted. The observable mutations are mostly caused by such drastic chromosomal alterations that several combinations are generally possible. The absolute frequency of mutations is considerably underrated by the mere registration of phenotypically identifiable mutations. This idea coincides very well with that of the 6*x*-wheat as a synthesis of three not completely identical but closely related genomes. It agrees with the limited phenotypical variation characteristic of the observed mutations. Thus the morphological mutations could almost without exception be characterized as changes in the length of the internodes of the straw or head with or without other pleiotropic effects, *e. g.*, on the glumes.

Interesting but also expected from the foregoing is the fact that the same phenotypes seem to occur spontaneously and artificially. There are even reasons to suppose a high degree of genotypic identity. In the present study it has not been possible to completely examine this analogy and the mechanism of the mutations. Thus, such an analysis has been concentrated on a certain type of mutation, *viz.* the speltoids and the related compactoids. This choice of test mutations was made for several reasons. The speltoids are phenotypically distinct, are true mutations that never can be confused with mere recombinations of different *vulgare* genotypes, and are induced by affecting only one single locus and are frequent. As yet, the study of all other mutations is quite summary. They have, however, been investigated to the extent that the idea of the mutation mechanism of a polyploid, given by the analysis of the speltoids and compactoids, could be proved of general application at least to 6*x*-wheat. A more extensive investigation of the non-speltoid mutations is, however, under way.

Since the Scandia III experiment was the one first started and gave the most distinct phenotypes, the detailed analysis of the different types of mutations has been mainly concentrated on this material.

1. SPELTOID MUTATIONS.

The speltoid mutation was first observed and described by NILSSON-EHLE (1917). It aroused great interest by its marked similarity to *Tr. Spelta* and its possibilities of revealing the relationship between this species and *Tr. vulgare*. It was stimulating because of its complicated ratios of segregation, its semi-dominant character, its striking pleiotropism (*cf.* Table 5) and its frequent spontaneous occurrence, which could even be detrimental to stock purity. Everything denoted a very complex mechanism of mutation.

With the new aspects on the speltoid problem that is a consequence of the artificial induction of mutations, it is important to stress that true speltoids may occur in different connections and here with different frequencies:

(i) *Spontaneous occurrence of speltoid mutations in homozygotisized lines.* — New cases of this category have been reported by NILSSON-EHLE (1917, 1920, 1921), VESTERGAARD (1919, 1921), PERCIVAL (1921), LINDHARD (1922), ÅKERMAN (1923), DUCELLIER (1923), LATHOUWERS (1930), DUMON (1931), ISHIKAWA (1934), FRANKEL and FRASER (1948), and ÅKERMAN and MAC KEY (1948). Occurrence of speltoid chimeras in pure strains has been proved by LINDHARD (*l. c.*), DUCELLIER (*l. c.*) and ÅKERMAN (1927).

In spite of this frequent verification of speltoid mutations appearing in fixed varieties, very few estimates are given as to their frequency. LINDHARD (*l. c.*) mentions 0,2 per cent and ISHIKAWA (*l. c.*) 0,08—0,3 per cent. GUSTAFSSON (1947) indicates a rate of 0,1—1 per cent without stating what kind of material it refers to or how it was determined. The estimates given by LINDHARD and ISHIKAWA are based on the number of speltoid plants in a certain material and are thus only of direct value as a control on the varietal purity. In addition, LINDHARD's material is less suitable for such an evaluation, as it includes progenies of normal plants that are direct descendants of het speltoids and related types of mutation. The only determination to be accepted, therefore, is the one based on the present material. From 0,6 (Scandia III) to 0,7 per cent (Rival) of speltoid mutations were here observed in the tested plant progenies of the control (*cf.* p. 87). These values seem, however, to be quite representative. They agree very well with the experiences of the Wheat and Oat Department of the Swedish Seed Association in extracting new strains from well established varieties of not too heterogeneous a parentage. With his long experience Dr. Å. ÅKERMAN asserts that the speltoid frequency of such a material generally reaches the ratio 1 : 200.

TABLE 5. *The pleiotropic effect of the speltoid mutation observed by a comparison to normal type.*

Character	Het speltoid	Hom speltoid	Author
Cell shape[1]	more elongated	more elongated	LI, HSIA and LEE, 1948
Leaf surface[2]	decreased	decreased — def. decreased	MAC KEY, unpublished
Plant height	increased	unchanged — decreased	NILSSON-EHLE, 1917
Tillering capacity	∾unchanged — decreased	decreased	UCHIKAWA, 1941
Head type	more lax, slender & pointed	def. more lax, slender & pointed	NILSSON-EHLE, 1917
Rachis: length	longer	unchanged — shorter	UCHIKAWA, 1941
brittleness	tough but progressively thinner	tough but progressively thinner brittle	WATKINS, 1928 PHILIPTSCHENKO, 1929
Spikelet number	slightly lower	lower	KAJANUS, 1923
Glume: length	slightly shorter	shorter	NILSSON-EHLE, 1917
texture	slightly thicker & tougher	thicker & tougher	WATKINS, 1928
face	slightly flatter & adherent	flatter, fitting closely to the grain	NILSSON-EHLE, 1917
tip	slightly truncated	truncated	»
keel	more developed	strongly developed	»
collar at base	well marked	very well marked	WATKINS, 1928
nervation	more developed & raised	strongly developed & raised	»
chlorophyll	more concentrated along the nerves	highly concentrated along the nerves	NILSSON-EHLE, 1917
Lemma: awnedness	unchanged — awnletted	unchanged — awned	NILSSON-EHLE, 1921
Pollen size	unchanged — varying	unchanged — smaller	MAC KEY, unpublished
Maturity	slightly delayed	delayed	NILSSON-EHLE, 1917
Leaf colour	unchanged — faintly pale green	pale green	LI, HSIA and LEE, 1948

[1] Only verified for epidermal cells and stomata; [2] ditto for flag leaf.

(ii) *Spontaneous occurrence of speltoid mutations in crosses.* — Speltoids often are a secondary consequence of intraspecific crosses in *Tr. vulgare*. This circumstance has been observed by KAJANUS (1918, 1923), NILSSON-EHLE (1920, 1921), LEIGHTY and BOSHNAKIAN (1921), PHIPPS and GURNEY (1932), LOVE (1940 a), ÅKERMAN and MAC KEY (1948), and JUNGFER (1953). The same experience holds for the speltoid chimeras, according to ÅKERMAN (1920, 1927) and KAJANUS (*l. c.*). Further, true speltoid mutations are not infrequent in interspecific crosses including *Tr. vulgare*. Such an occurrence has been reported by PHILIPTSCHENKO (1929), VASILJEV (1929), MICZYNSKI (1930), CAMARA (1936), KATTERMANN (1936/37, 1937), and ELLERTON (1939). The cross *Tr. vulgare*×*Tr. turgidum* may be considered as an exceptional case, since about 10 per cent of the F_2 are segregates of *Spelta* appearance. According to KAJANUS (1923, 1937), WATKINS (1927, 1928, 1930, 1940) and GRANHALL (1943), these so-called *speltoides* should be identical or closely related to the speltoid mutations.

As to the frequency of speltoid mutations in crosses, only those including *vulgare*×*vulgare* are of interest in this connection. Here, too, evaluations of the frequency are rare but LEIGHTY and BOSHNAKIAN (*l. c.*) mention 1—3 per cent and KAJANUS (*l. c.*) 0,3—1,9 per cent. These determinations are also based on the number of observed speltoid plants and do not consider the number of events of the mutation. KAJANUS, however, gives so detailed a report of his crosses that it is possible to determine the actual frequency of the mutation in his material:

No. of crosses	Generation	No. of plant progenies	Ditto with speltoids	Mutation frequency, %
10	F_2	47	24	51,1
3	F_3	722	135	18,7
1	F_4	89	15	16,9

In comparison with the above given figures of 0,6—0,7 per cent for established varieties, the frequency values calculated here clearly show that the speltoids occur more readily in a heterozygous than in a homozygous genotype. The data given by KAJANUS can be verified by cross material from the Wheat and Oat Department of the Swedish Seed Association, where Dr. Å. ÅKERMAN particularly made such analyses in a routine fashion from 1916 to 1920. There seems to be no doubt, however, that the frequency of speltoid mutations is dependent on the genotypical difference of the two parents. Closely related varieties generally give crosses with a much lower speltoid frequency than those from

widely separated types. KAJANUS' crosses should perhaps be put in the latter category rather than in the former.

(iii) *Induction of speltoid mutations by external influences.* — It is definitely shown that the speltoid mutability may be accelerated not only by such strong mutagens as X-rays (DELAUNAY, 1930, 1931, 1932, 1934; SAPEHIN, 1934, 1935; GUSTAFSSON, 1941; FRÖIER, 1946; MAC KEY, 1952; ONUFRIJCHUK, 1953), radiophosphorus (ARNASON, PERSON and NAYLOR, 1952) and fast neutrons (MAC KEY, 1952). Electric current (BYNOV, 1938 a and b; PIROVANO, 1939), heat shocks (SCHKWARNIKOW, 1936), and even mere storing of the seed (SCHKWARNIKOW, 1937, 1939) also work in the same direction. In addition, E. NILSSON (1934) obtained some indication that the physiological upset caused by bunt (*Tilletia*) may induce speltoid mutations, and MC FADDEN (quoted from HUSKINS, 1946) has evidence that frost may induce them during the flowering period.

The size of the frequency of induced speltoids varies considerably with mutagen, dose and method of application. In extreme cases, 40—50 per cent of the surviving plants of the first generation of treatment show speltoids in their offspring.

It is thus evident that the spontaneous speltoid frequency of a »pure line» may be considerably increased with a change to pronounced heterozygosity or by induced cytological disturbances. With the vivid discussion of identity between spontaneous and induced mutability as a background, it would be of interest to find out if the same genotypes are represented among speltoids of different origin and if the speeding-up processes in the two cases are identical in their end results.

The answer can only be given, however, after a detailed revision of the speltoid problem. Already NILSSON-EHLE (1921) classified the spontaneous speltoids as to their ratio-type and homozygous phenotype in four different groups which could be characterized in the following way:

	normal :	het speltoid	: hom speltoid	
Partial A-type	1 :	$< 2 — > 1$	: < 1;	beardless hom.
A-type	1 :	$< 2 — > 1$	: < 1;	bearded hom.
B-type	1 :	3,5—10	: $< 0{,}1$;	» »
C-type	1 :	$\leqq 1$	: $< 0{,}2$;	» »

The different ratio-types can mathematically be explained by the test cross het speltoid×normal, reciprocally, or het speltoid×hom speltoid, reciprocally, with the same effect. The interrelation on the female side between functioning normal and speltoid gametes is revealed in F_1, if the heterozygote is used as mother. The corresponding in-

TABLE 6. *A computation of test crosses het speltoid×normal (or hom speltoid), reciprocally, illustrating the interrelation of functioning normal and speltoid gametes.*

Speltoid type according to author	Homozygote used in the cross	No. of F_1 plants after the het speltoid used as				Interrelation of functioning normal and speltoid		Ratio-type of the het speltoid		Author
		mother		father						
		het	hom	hom	het	egg cells	pollen	expected	observed	
						I. *Deficiency-speltoids* ($2n = 42$):				
A	normal	9	10	10	7	1 : 1*	1 : 0,7*	1 : 1,7 : 0,7*	1 : 1,5 : 0,78*	UCHIKAWA, 1941
A	speltoid	7	5	5	8	1 : 1*	1 : 0,6*	1 : 1,6 : 0,6*	»	»
A	speltoid	31	34	10	39	1 : 1	1 : 0,25	1 : 1,25 : 0,25	1 : 1,24 : 0,26	ÅKERMAN and MAC KEY, 1948
A	normal	25	25	126	22	1 : 1	1 : 0,17*	1 : 1,17 : 0,17*	1 : 1,16 : 0,13	ÅKERMAN, 1923
C	normal	7	9	19	2	1 : 1	1 : 0,1	1 : 1,1 : 0,1	1 : 1,1 : 0,06	UCHIKAWA, 1941
C	speltoid	7	6	2	21	1 : 1*	1 : 0,1*	1 : 1,1 : 0,1*	»	»
C	normal	21	26	72	3	1 : 1*	1 : 0,04*	1 : 1,04 : 0,04*	1 : 1,1 : 0,01	SMITH *et al.*, 1949
C	normal	7	9	29	0	1 : 1*	1 : 0,0*	1 : 1,0 : 0,0*	1 : 1:0 : 0,0	NILSSON-EHLE, 1917
						II. *Monosomic speltoids* ($2n = 41$):				
B	speltoid	2	7	0	9	1 : 3,5*	1 : 0,0*	1 : 3,5 : 0,0*	1 : 3,1 : 0,0*	NILSSON-EHLE, 1921
B	normal	63	22	90	0	1 : 3*	1 : 0,0*	1 : 3,0 : 0,0*	1 : 3,7 : 0,0*	SMITH *et al.*, 1949
B	normal	23	6	15	3	1 : 4	1 : 0,2	1 : 4,2 : 0,8	1 : 4,5 : 0,05*	UCHIKAWA, 1941
B	speltoid	2	9	2	11	1 : 4*	1 : 0,2*	1 : 4,2 : 0,8*	»	»
B	normal	90	24	200	10	1 : 4	1 : 0,05	1 : 4,05 : 0,2	1 : 4,23 : 0,06	ÅKERMAN and MAC KEY, 1948
B	normal	195	14	103	0	1 : 14	1 : 0,0*	1 : 14,0 : 0,0*	1 : 8,6 : 0,0*	LINDHARD, 1922

* =Not given by the cited author.

formation about the male side is given with the heterozygote as father. The results of such analyses are computed in Table 6. They are all based on spontaneous speltoids.

As evident from the table, the speltoid pollen is always characterized by an underrepresentation at fertilization, while the egg cells either show the normal ratio 1 : 1 or a considerable overrepresentation of the speltoid type. This queer behaviour to begin with, appeared more than complex, and remained very difficult to interpret as long as no cytological examinations were made.

Omitting the historical development (*cf.* OEHLER, 1930; HUSKINS, 1946), the different gamete interrelations at fertilization may with our present knowledge be explained by the following observations:

(i) The functioning ability of the egg cell is not, or but very slightly, influenced by the speltoid mutation, while this always seems to handicap the pollen at fertilization.

(ii) A mutation, phenotypically classified as a speltoid, may be caused either by a point mutation, by a larger deficiency, or by the total loss of a certain chromosome.

(iii) Generally speaking, the functioning efficiency of the speltoid-bearing pollen is conversely proportional to the size of the deleted segment (*cf.* p. 121 and Fig. 76).

(iv) The univalent of the monosomic het speltoids shows lagging, is thus easily excluded from the daughter nuclei and causes an increased frequency of (n-1)-gametes, *i. e.*, speltoid-bearing gametes.

From the above it may be clear that the speltoid mutation is induced by a change of one or a very few loci, while the ratio-type is dependent on the loss of chromosomal material. This mechanism and its consequences allow the speltoid B-type to be clearly separated from the other groups. These are all to be considered as deficiencies (or point mutations) in the particular chromosome without the centromere being lost. As to the ratio-type of the different deficiency-speltoids, the partial A-type should be interpreted as the smallest possible change, the common A-type as an intermediate state and the C-type as the largest possible loss in that particular arm of the chromosome. Justifying in this manner the old grouping of NILSSON-EHLE by present cytological facts must, however, presuppose that the speltoid mutation of type A and C always is a simple deficiency (or a point mutation). Further, the size of this deficiency should alone regulate the functioning ability of the pollen. Such an idea of the cytological background to all A- and C-speltoids is also supported in the latest papers on speltoid mutations

(HUSKINS, 1946; HUSKINS and SANDER, 1949; SMITH, HUSKINS and SANDER, 1949; SMITH, 1951; JUNGFER, 1953). In these papers the old grouping has also been accepted. The mechanism behind the mutation, however, is not always so simple (see below). More complicated processes with substitution of chromosomal segments and thus with apparent compensatory effects act in such a manner that the size of the deficiency in the chromosome regulating the speltoid character not always determine the ratio-type. An entirely continual transition from one type of segregation to another and, as will be later shown, a very unfortunately chosen dividing line, make the grouping into type A and C less justified. It is definitely more natural to bulk them into one group and then give them a more descriptive name, *e. g.*, *deficiency-speltoids* (conveniently abbreviated to *def-speltoids*). If a subgrouping is still desired, this should not include the two types A on one hand and the type C on the other. The boundary should be drawn with respect to the beardedness of the hom speltoid, *viz.* the partial type A on one hand as a beardless def-type and other A- and C-types on the other hand as a bearded def-type. Only this distinction can be made consistently. The monosomic speltoid (*monosome-type* with a nullisomic homozygote) is distinctly separated.

A. SPELTOID MUTATIONS OF DEFICIENCY-TYPE.

As explained above, the speltoid types A and C are included in the conception of def-speltoids. This group is characterized by a normal chromosome number ($2n=42$). If no failure occurs in meiosis, this means gametes of $n=21$ and a normal : speltoid proportion of 1 : 1 in the heterozygote. The deviation from the expected 1 : 2 : 1 segregation is mainly determined by the different efficiency of normal and speltoid-bearding pollen to function at fertilization, which regulates the deficit of het and hom speltoids. Apart from the most drastic deviations, the type of segregation is characterized by the number of heterozygotes equalling the sum of the two homozygotes.

Speltoids of this ratio-type have been observed in pure strains as well as in cross materials. As evident from Tables 7 and 10 and Fig. 76, they also may be artificially induced. Beardless and bearded def-speltoids of all gradations in ratio-type may occur.

Before discussing the origin and cytologic constitution of the def-speltoids, some cytogenetic data should be given about the speltoid mutation. As stated above, the appearance of the speltoid character is regulated by a particular pair of chromosomes, denoted by WINGE

TABLE 7. *Analysis of induced speltoid mutations, Scandia III, 20.000 r.*

X_2 line 1945—959—	Segregation in offspring of the het speltoid: absolute values: normal type	het speltoid	hom speltoid	subcompactoid	Total	ratio-type: norm : het : hom : subc	Mutation mechanism
	I. *Deficiency-speltoids (2n = 42);*						
	a) with the homozygote beardless:						
295	257	474	182	—	913	1 : 1,8 : 0,71	def. or gene mut.
153	307	556	248	—	1111	1 : 1,8 : 0,81	def-dupl.
174	363	526	111	—	1000	1 : 1,4 : 0,31	»
298	368	419	78	—	865	1 : 1,1 : 0,21	»
	b) with the homozygote bearded:						
25_I	97	181	40	—	318	1 : 1,9 : 0,41	»
34	161	296	101	—	558	1 : 1,8 : 0,63	» [1]
7	202	332	85	—	619	1 : 1,6 : 0,42	deficiency
222	136	170	21	—	327	1 : 1,3 : 0,15	def-dupl.
25_{II}	380	429	53	—	862	1 : 1,1 : 0,14	deficiency
93_I	329	369	55	6	759	1 : 1,1 : 0,17 : 0,02	def-dupl.
26_I	290	310	31	—	631	1 : 1,1 : 0,11	deficiency [2]
365	157	167	—	—	324	1 : 1,1 : 0,00	»
209	163	172	6	—	341	1 : 1,1 : 0,04	»
22	617	623	4	—	1244	1 : 1,0 : 0,01	» [3]
165	161	161	26	—	348	1 : 1,0 : 0,16	»
72	215	210	—	—	425	1 : 1,0 : 0,00	»
319	227	220	9	—	456	1 : 1,0 : 0,04	»
219	225	218	8	—	451	1 : 1,0 : 0,04	def-dupl.
184	267	254	3	—	524	1 : 1,0 : 0,01	deficiency
427	186	176	—	—	362	1 : 0,9 : 0,00	»
141	138	125	1	1	265	1 : 0,9 : 0,01 : 0,01	def-dupl.
178	141	127	—	—	268	1 : 0,9 : 0,00	deficiency
18	245	216	10	—	471	1 : 0,9 : 0,04	»
402	154	135	20	—	309	1 : 0,9 : 0,13	def-dupl.
257	176	154	5	—	335	1 : 0,9 : 0,03	deficiency
154	215	188	—	—	403	1 : 0,9 : 0,00	»
122	208	143	1	—	352	1 : 0,7 : 0,00	def-dupl. [1]
93_{II}	247	104	—	—	351	1 : 0,4 : 0,00	deficiency
Σ	6632	7455	1098	7	15192	1 : 1,1 : 0,16	
	II. *Monosomic speltoids (2n = 41):*						
35	73	288	2	1	364	1 : 3,9 : 0,03 : 0,01	monosomy
25_{III}	95	429	—	1	525	1 : 4,5 : 0,00 : 0,01	»
350	47	220	—	—	267	1 : 4,7 : 0,00 : 0,00	»
156	55	260	1	—	316	1 : 4,7 : 0,02 : 0,00	»
26_{II}	46	245	1	—	292	1 : 5,3 : 0,02 : 0,00	»
42	38	216	—	1	255	1 : 5,7 : 0,00 : 0,03	»
28	36	250	1	1	288	1 : 6,9 : 0,04 : 0,04	»
36	94	702	4	5	805	1 : 7,5 : 0,04 : 0,05	»
25_{IV}	107	897	1	1	1006	1 : 8,4 : 0,01 : 0,01	»
419	36	326	2	2	366	1 : 9,1 : 0,05 : 0,05	»
Σ	627	3833	12	12	4484	1 : 6,1 : 0,02 : 0,02	

[1] Followed by monosomy of the non-*Q* bearing chromosome; [2] = misdivision, isochromosome of the short arm of IX; [3] + an extraneous def-dupl.

(1924) with »C», and by SEARS (1944) with »IX». [It is a principle to designate single factors with letters and chromosomes with Roman numerals. This rule will be followed in the present paper in spite of the priority of the »C» denotation. The speltoid nomenclature will thus be inserted in the complete registering of the 21 pairs of wheat chromosomes. Various derivatives of chromosome IX will here also be denoted according to SEARS (1952 a and b). The earlier proposals of HUSKINS (1946) will not be considered, since they are based on the symbol C.] This chromosome IX is submedian, with two factors located in its long arm, one regulating the specific speltoid character (*Q*; *cf.* pp. 159—160), the other one the beardedness (B_1). NILSSON-EHLE's (1927) analysis of the cross bearded normal×beardless hom speltoid, with a gamete relation in the F_2 of $1:2{,}8:2{,}8:1$, shows that the factors *Q* and B_1 are at least 26 crossing-over units apart. The two factors have an inhibiting function, for which reason the true speltoid and beard promoting genes were presumed to be found in other chromosomes. WINGE (1924) thinks that these factors are present in a chromosome homoeologous to IX, an idea earlier generally accepted. [The term homoeologous is coined by HUSKINS (1931) for chromosomes that are phylogenetically alike in a polyploid series but are now only similar.] According to this hypothesis of WINGE, the normal *vulgare* phenotype is the result of the two pairs of chromosomes balancing each other. A deficit of IX, *i. e.*, a deficit of inhibitors, will cause a speltoid phenotype. A surplus will give the dense-eared compactoid type which thus is to be considered as the result of an overdose of suppressors.

How does this shift of balance occur when def-speltoids are produced? Even if one does not consider the hypotheses based solely on genetic analyses, such as that of partial heterogamy (NILSSON-EHLE, 1921) nor that of the speltoid being a rare product of recombination (KAJANUS, 1923), there are still not less than seven distinctly different suggestions for explaining the def-type or a subgroup of it. These explanations, all based on spontaneous material, are:

(1) *Fragmentation followed by a simple deficiency.* — The speltoid suppressors disappear through a loss in the long IX-arm (NILSSON-EHLE, 1920; HUSKINS, 1927, 1933, 1946; MÜNTZING, 1930; WATKINS, 1930; NISHIYAMA, 1933; UCHIKAWA, 1934, 1936, 1941, 1946; LOVE 1940 a; CAMARA and RODRIGUES, 1946; ÅKERMAN and MAC KEY, 1948; HUSKINS and SANDER, 1949; SMITH, HUSKINS and SANDER, 1949; SMITH, 1951; JUNGFER, 1953). This explanation was given for both type A and C in the old classification scheme.

(2) *Substitution of chromosomes.* — At homoeologous pairing, chromosome IX may be replaced by its not completely identical partner. As this lacks the suppressors, they will be lost (WINGE, 1924; HUSKINS, 1927, 1928). This explanation has only been proposed for the non-partial type A.

(3) *Deficiency-duplication.* — Homoeologous pairing followed by crossing-over is supposed, the particular factors being included in the translocated segments. Secondary products of this process may show a deficit of the suppressors (WINGE, 1924; HUSKINS, 1927, 1933, 1941; ELLERTON, 1939). The explanation is suggested for type A.

(4) *Gene mutation.* — A clear proposal to explain the speltoid as a simple gene mutation has never been made. On the other hand, it was suggested that the speltoid could occur from a simultaneous mutating of a whole block of genes, with the normal type, the beardless and the bearded speltoid as some kind of allelomorphs (NILSSON-EHLE, 1920; UCHIKAWA, 1934, 1936, 1941, 1946). This explanation is applied to type A.

(5) *Aneuploidy.* — An unbalance between chromosome IX and its homoeologue may occur: (a) by the latter being duplicated, *i.e.*, the speltoid-bearing gamete should have $n=22$ (HUSKINS, 1927, 1928, 1933; HÅKANSSON, 1930; MÜNTZING, 1930); (b) by a loss of chromosome IX and a speltoid gamete of $n=20$ (VASILJEV, 1929; VASILJEV and KAMENIK, 1935). Both explanations have been suggested for type C.

(6) *Duplication through the production of isochromosomes.* — Misdivision of the homoeologous chromosome is considered to cause an isochromosome with a duplicated set of the beard- and speltoid-promoting factors (CAMARA and RODRIGUES, 1946). This explanation for an underrepresentation of the suppressors has only been proposed for the type C.

(7) *Position effect.* — If the suppressors are translocated to another chromosome, they should lose their inhibiting ability without directly disappearing (CAMARA, 1936; CAMARA and RODRIGUES, 1946). The explanation is suggested for type C.

With our present knowledge, all the above-mentioned hypotheses cannot be accepted. The three last-mentioned must definitely be discarded. VASILJEV's (*l. c.*) observation that the chromosome IX was lost without being substituted, must be explained by a change from def- to monosome-type. This phenomenon has frequently been observed as a direct consequence of the decreased ability of def-IX to pair at meiosis, and a subsequent genetic analysis of this particular case proved this explanation to be valid (HUSKINS and SANDER, 1949, p. 341). The other

form of aneuploidy proposed may be thought to give a ratio-type characteristic of the def-speltoids, but this suggestion, based on chromosome addition, cannot be accepted either, however. None of SEARS' nearly complete series of trisomics and tetrasomics, including the chromosomes V and XVIII which are homoeologous to IX, have speltoid appearance (personal communication). A particular chromosome balancing the *Q*- and B_1-factors of IX does not exist. Thus the significance of a supposed trisomy has been misunderstood. The hypothesis is further disproved because all reports on trisomy can be explained as mere misinterpretations of the heteromorphic bivalent IX : def-IX (*cf.* HÅKANSSON, 1930; NISHIYAMA, 1933; HUSKINS, 1946).

From SEARS' observations it is further evident that the occurrence of an isochromosome in a speltoid cannot be given the significance cited above. A non-IX isochromosome will never provoke a true speltoid phenotype. An isochromosome in a purebred def-speltoid may, however, appear as a result of the particular type of deficiency that misdivision is. The isochromosome of IX must be made up by its short arm (*cf.* mutant 26_I of Table 7). An isochromosome of an intact long arm of IX can never appear in a speltoid, which is a direct contradiction of ONUFRIJCHUK's interpretation (1953).

The third explanation to be discarded is that based on a supposed position effect. It will be shown below that the *Q*-factor will retain its suppressing ability even in a translocated position (*cf.* pp. 110—111).

No definite arguments have as yet been presented against the four remaining hypotheses. Only one of them, the deficiency theory, is conclusively proved as one explanation, however. The hypotheses of chromosome substitution and deficiency-duplication were put forward because of the observations of trivalents or tetravalents in the M I of het and hom speltoids. Even if these observations were never proved to be directly associated to the speltoid phenomenon, HUSKINS and co-workers in their recent papers (1946, 1949, 1951) mistakenly minimize them and their purported significance. The same must also be said about single speltoid mutations as being more or less complicated gene mutations. As long as the true nature of a point mutation remains unknown, we will never be able to disprove that beardless speltoids in extreme cases cannot be true gene mutations. This explanation is, however, not valid where the hom speltoids are bearded. The great distance between *Q* and B_1, the absolutely parallel mutating of these factors and the non-occurrence of any separation by crossing-over, all argue in favour of a segment loss.

From our present knowledge, the four explanations retained for the origin of def-speltoids seem to be the only ones to be considered. Thus, it should also be possible to fit in all artificially induced speltoids of the same ratio-type within the four supposed mechanisms of the mutation.

Considering the mode of action of X-rays and fast neutrons, numerous cases of fragmentation followed by a simple deficiency are expected to be found (*cf.* Table 7 and Figs. 58—62). Different degrees of deficiency in the long arm of chromosome IX have occurred in the different cases. The mere existence of a heteromorphic bivalent or two univalents of unequal size in certain metaphases I of the het speltoid is, however, not sufficient to prove that a simple deficiency is the initial cause of the mutation. Chromosome substitution as well as deficiency-duplication may result in similar heteromorphic 1_{II} or 2_{I}. In such a case these configurations will not be represented in all M I plates but alternate with $1_{III}+1_{I}$ and 1_{IV}. This has also happened in some of the induced speltoids. None of the speltoids examined, however, have arisen through chromosome substitution, which would mean frequent $1_{III}+1_{I}$ in the heterozygote and a tetravalent of four identical chromosomes in the homozygote. This negative result does not dismiss the idea of the substitution of whole chromosomes as a source of speltoid mutations, since this mechanism could hardly be accelerated by mutagenic treatments. If chromosome substitution really occurs as one explanation of speltoid induction, there is, however, reason to assume that it is with a very low frequency. One may possibly rule out this hypothesis, as does HUSKINS (1946). A more definite stand could be taken if SEARS' mono-IX was crossed with trisomics of the homoeologous chromosomes, *i. e.*, with tri-V and tri-XVIII, respectively. These crosses would give synthesized substitution speltoids whose functioning ability and ratio-type could be checked.

If the observed high frequency of multivalents is really closely associated to the speltoid character, deficiency-duplication must thus be the only or at least the most important explanation. To prove that this mechanism exists and is able to function in the production of speltoids, some detailed analyses will here be presented (*cf.* also KIHARA and NISHIYAMA, 1937; KOSTOFF, 1937; SMITH, 1948; MENZEL and BROWN, 1952). Since the result is somewhat different, if the primary cause is a simple or a reciprocal translocation (or a semi-homologous crossing-over), these alternatives should be treated separately. Fig. 18 illustrates the course of the process after an induced reciprocal trans-

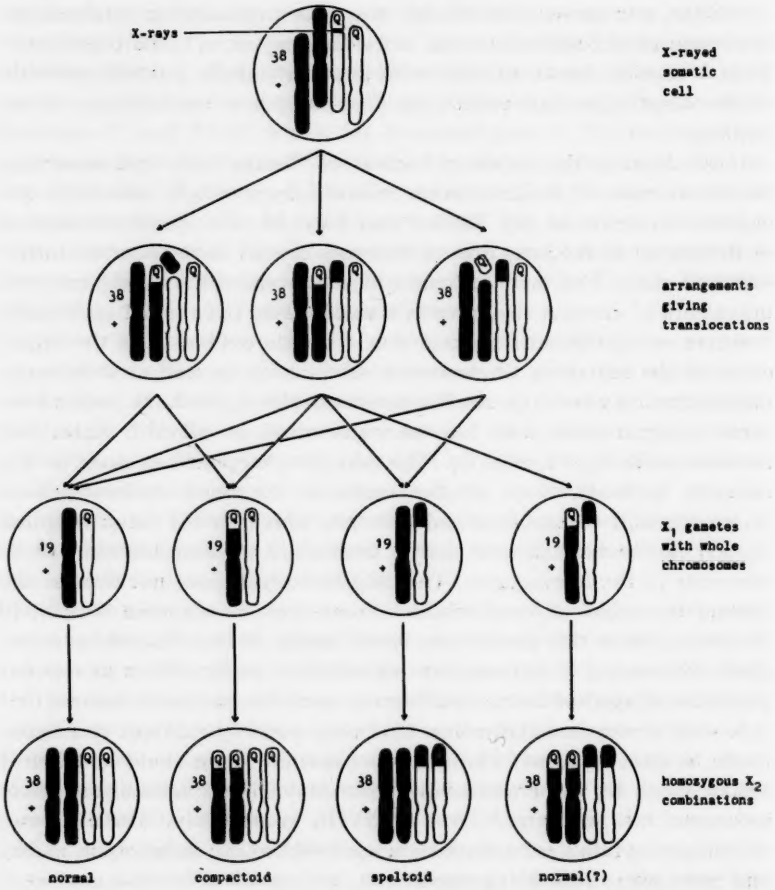


Fig. 18. Diagram of a translocation followed by deficiency-duplication. Chromosome IX (white), which includes the speltoid suppressing but compactoid promoting *Q*-factor, is supposed to be engaged.

location with the situation of the *Q*-factor consistently marked. The diagram also indicates the recombinations possible after a simple translocation. To avoid too complicated a diagram, the course of such a process was not completely followed up, however.

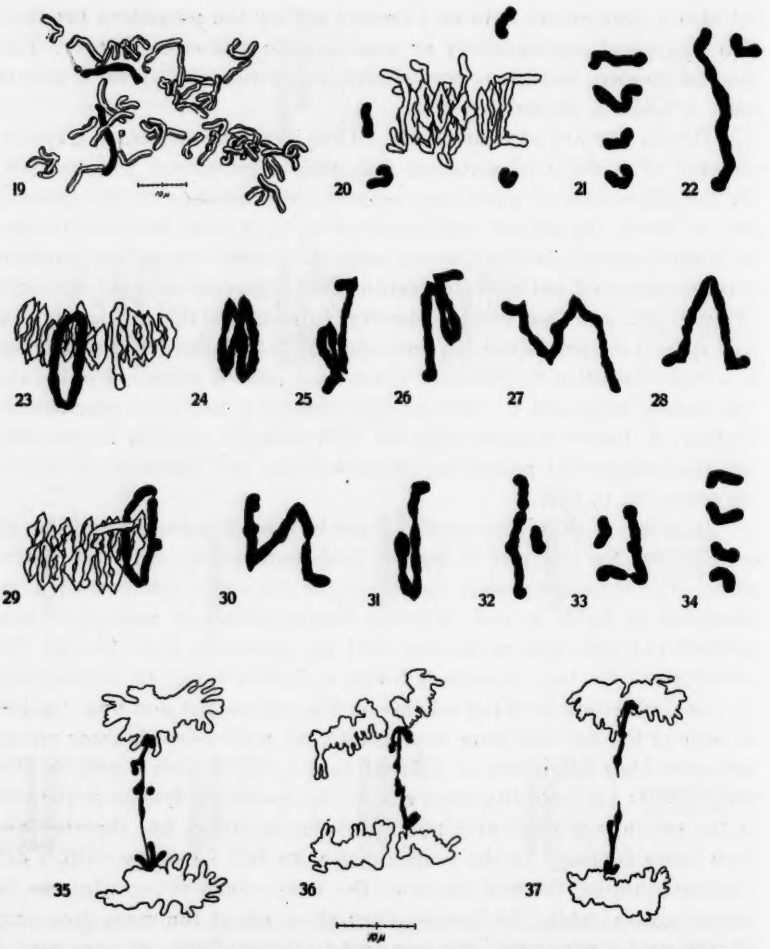
Considering the effect of the *Q*-factor to be unchanged in a translocated position (*cf.* hypothesis No. 7 of CAMARA), not only a speltoid

but also a compactoid is to be expected among the secondary products of a reciprocal translocation or semi-homologous crossing-over. This can also happen, because a mere change in position of *Q* never is able to cause a speltoid phenotype.

The X_2 line 174 of Scandia III, 20.000 *r* may be chosen as a typical example of a def-dupl-mutation following a reciprocal translocation. (In the discussion of particular mutants, the number of the progeny plot, in which the mutant was observed in X_2, is here consistently used for identification.) In this case not only the possible secondary products were all observed but also the translocated segments were of distinctly different size and thus easy to identify. In addition, this particular case is of special interest, since the hom speltoid is beardless. It thus belongs to a type classified by NILSSON-EHLE as a partial mutation only, and was hardly supposed to have so complicated a mutation mechanism. Further, it disproves admirably the idea that the speltoid suppressing and the compactoid promoting characteristics are regulated by different genes (*cf.* p. 159).

In order to study the mutant types in their purebred condition, all material for the analyses is derived from backcrosses with the mother strain. The cytological data necessary for the exact interpretation are presented in Table 8, and different configurations at meiosis of hom speltoid 174 and hom compactoid 174 are shown in Figs. 19—41. The ratio-type of the two mutants is found in Tables 7 and 12, respectively.

In conformity with the scheme on Fig. 18, the het and hom speltoid as well as the het and hom compactoid, all with $2n=42$, show an abnormally high frequency of 1_{IV} and $1_{III}+1_{I}$ (*cf.* Tables 3 and 8). The multivalents are not only observed in the heterozygotes, as in the case of the primary product of a reciprocal translocation, but they are also even more frequent in the homozygotes. In full harmony with a def-dupl-mechanism, the rod shape of the tetravalents is prevalent in the heterozygotes, while the homozygotes show about the same frequency of rods and rings (rods: 49,5 per cent in the speltoid, 56,7 per cent in the compactoid). Speltoid 174 has a considerably larger duplication than deficiency, while the reverse holds true for the compactoid. This condition explains the different frequency of multivalents of the two mutants. The tetravalents are easily recognized, especially in the speltoid, as being composed of chromosomes of unequal size (*cf.* Figs. 23—31, 38, 39). Together with the high frequency in the heterozygote of 1_{IV} against $1_{III}+1_{I}$, this rejects a substitution of whole chromosomes as being the cause of the mutation. The unequal size could not only be



Figs. 19—28, hom def-dupl-speltoid 174. — Fig. 19, root tip mitosis, $2n=42$, with the long translocated IX chromosomes easily identifiable. — Figs. 20—21, M I with 4_I. — Fig. 22, M I with $1_{III}+1_I$. — Figs. 23—28, M I with 1_{IV}.
Figs. 29—37, hom def-dupl-compactoid 174. — Figs. 29—31, M I with 1_{IV}. — Figs. 32—33, M I with $1_{III}+1_I$. — Fig. 34, M I with 4_I. — Fig. 35, T I brigde with a fragment. — Figs. 36—37, T I bridge without fragment.

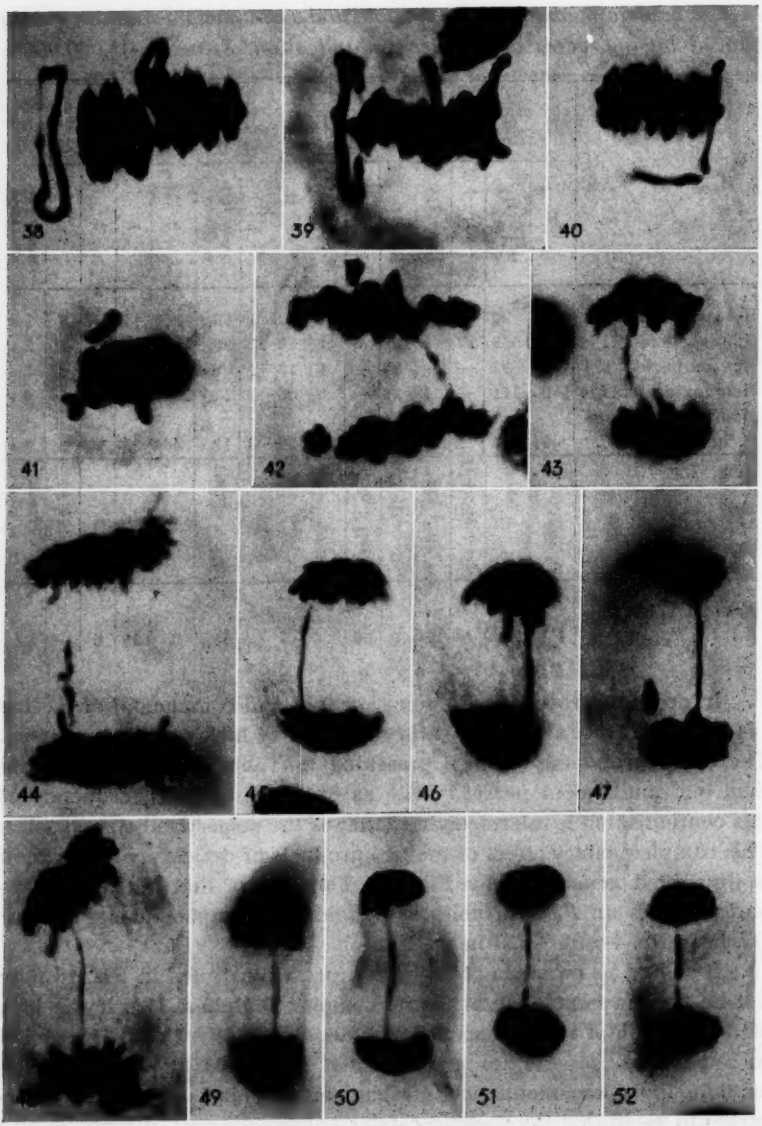
observed in the tetravalents but also in the rather common configuration of $20_{II}+2_I$, where the combination of one big and one small univalent occurs in about 30 per cent.

TABLE 8. *Meiotic analysis of a speltoid and a compactoid simultaneously induced by a process of deficiency-duplication. Scandia III, 20.000 r.*

Type	Metaphase I										No. of whole chromosome micronuclei in the tetrads				
	2_{II}	$1_{II}+1_{IV}$	$1_{II}+1_{IV}+2_I$	$1_{II}+2_{IV}$	$1_{II}+1_{III}+1_I$	$1_{II}+1_{III}+3_I$	$1_{II}+1_I+?$	$2_{II}+2_I$	$4_I-1_{0_I}$	1_{IV} or 1_{III}, % of total	0	1	2	$\wedge 2$	abnormal, % of total
Het speltoid 174	227	26	—	—	18	—	2	22	1	15,2	1248	44	18	3	5,0
Hom speltoid 174	1607	559	12	2	122	3	42	119	15	29,1	2660	60	29	6	3,4
Het compactoid 174	698	57	—	—	12	—	4	19	1	9,0	1876	53	11	2	3,4
Hom compactoid 174	1003	141	—	1	26	—	6	48	2	14,0	1832	58	17	1	4,0
Hom sp. 174 × hom comp. 174, F_1	404	45	1	—	19	—	6	39	4	13,1	400	22	10	3	8,0
	$2_{II}+1_I$ (long)	$2_{II}+1_I$ (short)	$1_{II}+1_{IV}+1_I$	$1_{II}+1_{III}$	$1_{II}+1_{III}+2_I$	$1_{II}+3_I$				1_{III}, % of total	0	1	2	$\wedge 2$	abnormal, % of total
Hom sp. 174 × null-IX, F_1	158	77	5	38	1	56				11,6	23	20	6	—	53,1

In order to prove that the hom speltoid and compactoid of X_2 line 174 are each other's complementary types, they have been intercrossed. The cytological analysis, also presented in Table 8, shows not more than one multivalent per M I-plate as expected and the frequency of this configuration is intermediate to that of the respective heterozygotes. This complementary effect of the two products of deficiency-duplication is illustrated genetically by Fig. 53. This shows that speltoid × compactoid gives an F_1 with neither speltoid nor compactoid appearance (*cf.* head No. 4 with No. 1 in the figure).

It should be pointed out that the process illustrated in Fig. 18 may not be the only explanation to the complex mutation 174, if the idea of the position of the true speltoid factors coincides with that presented in hypotheses (5) and (6) already cited (*cf.* p. 107). With this conception, the above-mentioned mechanism may just as well be interpreted to mean that the speltoid carries a duplicated set of markers and the compactoid none. Thus, chromosome IX should not necessarily be involved in the process. That this cannot be the explanation, however,


38
39
40
41
42
43
44
46
47
49
50
51
52

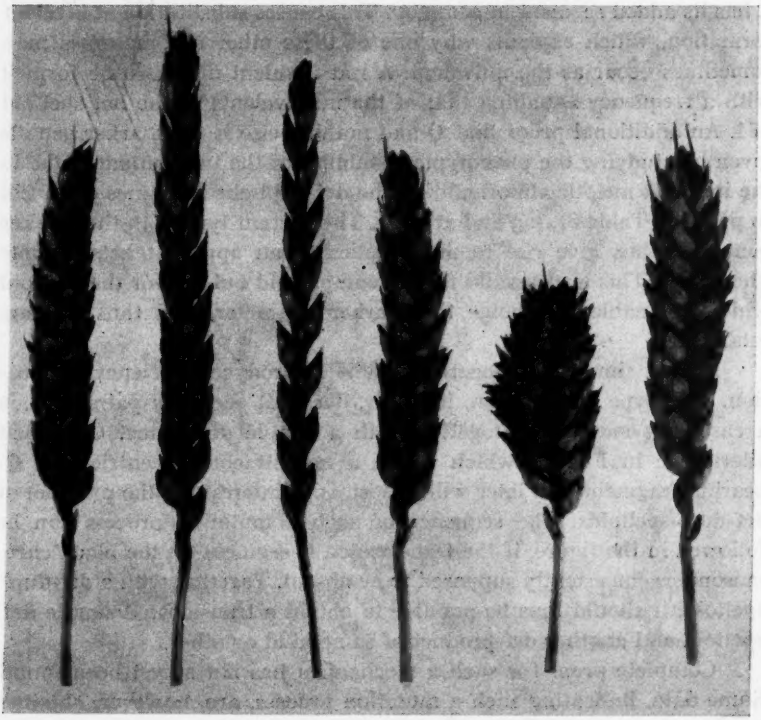


Fig. 53. Complementary deficiency-duplications. — From left to right: normal head of Scandia III, het speltoid 174, hom speltoid 174, F_1 between this and the following, hom compactoid 174, and subcompactoid 174.

can be shown, not only by SEARS' above-mentioned studies of trisomics but also by a cytological analysis of hom speltoid 174×nulli-IX (hom speltoid 35 used here). This control is presented in Table 8. The univalent of the 41-chromosome F_1 plants, which have the phenotype of the beardless hom speltoid, is the very big translocation chromosome. This must therefore be a changed IX without the *Q*-factor. It has, however, the ability to conjugate with that pair of chromosomes with which

Figs. 38—52. Meiotic configurations of various def-dupl-mutations. — Fig. 38, hom speltoid 174, M I with 1_{IV}. — Fig. 39, hom compactoid 174, M I with 1_{IV}. — Fig. 40, hom speltoid 153, M I with an uncommon type of rod 1_{IV}. — Fig. 41, speltoid 174× compactoid 174, F_1, M I with IX+translocated def-IX. — Figs. 42—47, A I and T I bridges of speltoid 298, 174 and 93_I. — Figs. 48—52, T II bridges of compactoid 174 and speltoid 174 and 93_I.

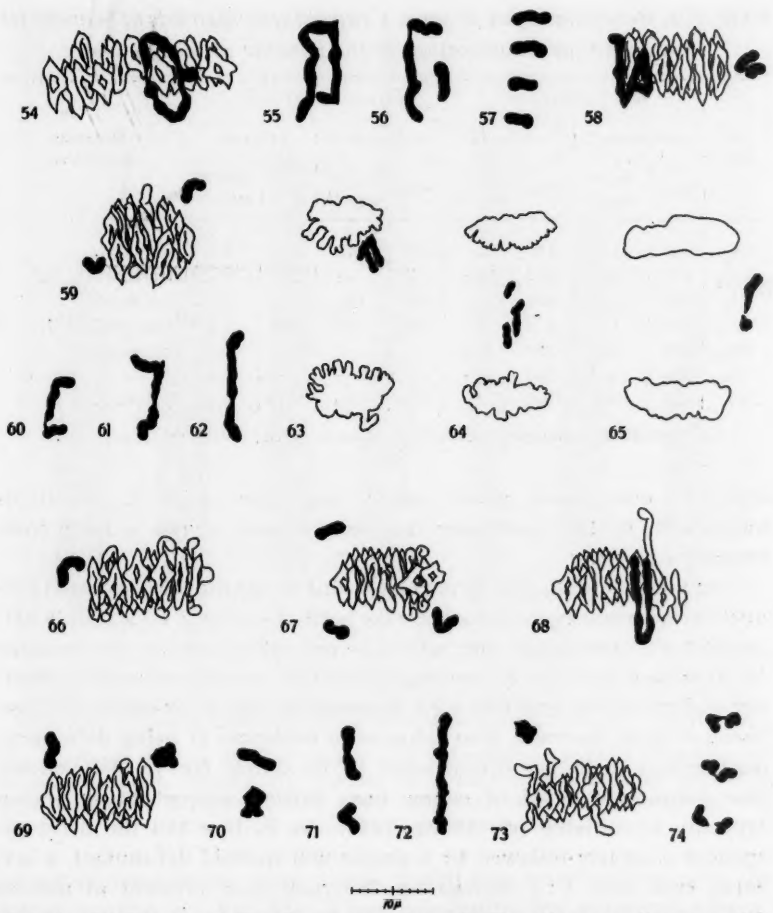
it has its added segment in common. This causes substitution at bivalent formation, which explains why one of these other chromosomes may sometimes occur as the univalent. A rod trivalent may also be formed with a frequency equalling that of the multivalents of the het speltoid 174. An additional proof that *Q* and nothing else is the marker gene is given by studying the phenotypical stability of the two mutants. Due to the inherent meiotic abnormalities, the def-dupl-chromosomes often fail to pair (*cf.* Table 8), lag and are lost. The mutant type with the marker gene will thus give rise to monosomics of an apparent heterozygote phenotype. This is also valid for the compactoid but not for the speltoid which is unable to change the marker character, and thus appears established.

Even a simple translocation is able to produce deficiency-duplication. This type of mutation, however, does not occur by pairs in such a case, but may occur together with a simple deficiency. Only that alternative in Fig. 18 which shows a non-attached, acentric and *Q*-bearing fragment that later will be lost, is of interest for the problem of def-dupl-speltoids. The sequence of such a mutation process can be followed in the figure, if the translocated *Q*-segment on the black chromosome is consistently supposed to be absent. Together with a def-dupl-speltoid, it should thus be possible to obtain a translocated simple def-speltoid and another def-product of some kind or other.

Complete proof for such a mechanism has not as yet been found. Some data, indicating such a mutation process, are, however, obtained for the Scandia III material (*cf.* Tables 7 and 9 and Figs. 54—57 and 62).

Thus, speltoid 93_{I} has been shown to be a def-dupl-type, while speltoid 93_{II} is a simple def-type with a heteromorphic bivalent but without an abnormal formation of multivalents. The extreme mode of segregation and the very high frequency of micronuclei suggest more than an uncomplicated deficiency. It may be logical to suspect a translocated deficiency.

X_2 line 25 is another case. Speltoid 25_{I} is of def-dupl-type, while 25_{II} is a simple def-type and probably a translocated one. Such an interpretation is supported by the fact that the two def-speltoids are followed by two not completely identical monosome-speltoids. These must have occurred by the loss of the changed chromosome IX. It then seems natural to interpret the difference in ratio-type by the remaining chromosome set being complete in one case and homozygous for a deficiency in the other. In the latter case the balance would probably be



Figs. 54—74. Meiotic configurations of various speltoids and compactoids induced by X-rays in Scandia III. — Figs. 54—57, beardless hom def-dupl-speltoid 153, M I with 1_{IV}, $1_{III}+1_{I}$ or 4_{I}. — Fig. 58, het def-speltoid 22 with an extraneous def-dupl-mutation, M I with $18_{II}+1_{IV}+IX+$def-IX. — Fig. 59, het def-speltoid 72, M I with $20_{II}+IX+$def-IX. — Figs. 60—62, heteromorphic 1_{II} of het speltoid 22, 72 and 93_{II}. — Figs. 63—65, het monosome-speltoid 25_{IV}, T I with non-disjunction or misdivision. — Figs. 66—68, trisome-subcompactoid 80, M I with $21_{II}+IX$, $20_{II}+3\,IX_{I}$ or $20_{II}+IX_{III}$. — Figs. 69—71, iso-subcompactoid 25_{II}, M I with IX+iso-IX. — Fig. 72, ditto, heteromorphic IX : iso-IX bivalent. — Fig. 73, iso-subnormal 25_{II}, M I with $20_{II}+$iso-IX. — Fig. 74, ditto, iso-IX's showing tendency to misdivide.

TABLE 9. *Meiotic analysis of some X-ray induced speltoids of Scandia III with a simple translocation as the possible primary cause.*

X_2 line	Het speltoid:				Hom speltoid:				Mutation mechanism
	metaphase I		tetrads		metaphase I		tetrads		
	total	1_{IV} or 1_{III}, %	total	abnormal, %	total	1_{IV} or 1_{III}, %	total	abnormal, %	
93_I	446	24,2	418	7,2	154	25,9	154	8,2	def-dupl.
93_{II}	170	1,8	272	10,2	—	—	—	—	translocated def.
25_I	443	16,9	432	11,8	352	19,6	563	6,6	def-dupl.
25_{II}	314	1,0	371	7,3	181	1,1	261	1,9	translocated def.
25_{III}	381	3,4[1]	530	33,9	—	—	—	—	monosomy
25_{IV}	299	2,0[2]	395	40,0	—	—	—	—	»
153	494	9,3	1532	2,9	703	10,8	1135	5,2	def-dupl.

[1] 1_{III} including the single chromosome = 2,6 per cent; [2] ditto = 1,7 per cent.

disturbed even more, which seems reasonable, since the def-dupl-mechanism of this particular mutation process shows a high compensatory effect.

In neither the X_2 line 25 nor the X_2 line 93 has the third expected product of the primary mutation process been observed, *i. e.*, a simple def-product with phenotype and ratio-type not determined by chromosome IX. It is true that line 25 has segregated two compactoids and a short-strawed mutation, and line 93 a dense-eared and a lax-eared off-type. None of them, however, give cytogenetic evidences of being deficiency-duplications including chromosome IX or simple non-IX deficiencies. The def-mutant could of course have existed without being phenotypically identifiable (*cf.* SEARS, 1944). In X_2 line 153 the def-dupl-speltoid is clearly followed by a simple non-speltoid def-mutant, a lax-eared type with 1 : 1 segregation, heteromorphic bivalent at meiosis and lacking the homozygote. Here, on the other hand, the simple def-speltoid is lacking.

Besides the definite proof that all def-speltoids can by no means be considered as simple deletions, the above described mechanism of deficiency-duplication offers a great deal of interest. It presupposes a process that can only be expected to give vital end products in connection with polyploidy or other kinds of reduplication. It means a process of mutation able to produce more than one mutant type, which is of greatest importance in a material where a pronounced grouping of the induced mutations has been found. It gives great possibilities of

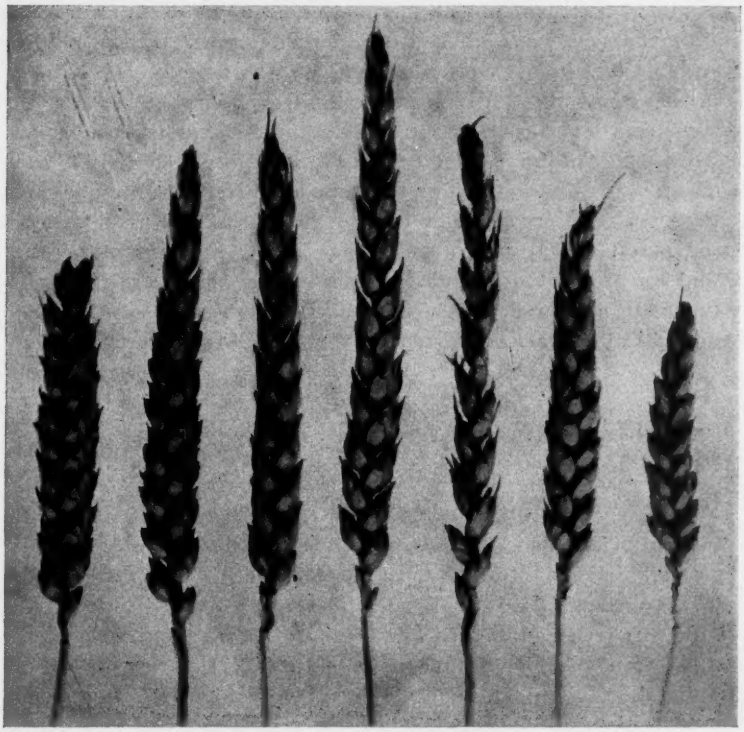


Fig. 75. Heads showing different phenotypical expressions of speltoid def-dupl-mutations in one strain (Scandia III). — From left to right: normal head, het speltoid as gene mutation or minute deficiency, other heads representing het def-dupl-speltoids (No. 5 with an extraneous def-dupl-mutation).

recombinations in the germ plasm, where true translocations give the initial impulse (*cf.* Fig. 75). When considering the numerous observations of multivalents in spontaneous speltoids (WINGE, 1924; HUSKINS, 1927, 1928, 1933; CAMARA, 1936; KATTERMANN, 1938; CAMARA and RODRIGUES, 1946), it thus seems highly probable that def-dupl-speltoids also occur in nature. Here, however, the semi-homologous crossing-over surely gives the main impulse. The recombining ability will thus probably be more restricted than in an induced material. In relation to the simple deletion, the deficiency-duplication may mean a positive, an indifferent, or a negative arrangement with respect to vitality. Thus, Table 7 presents def-dupl-speltoids which show either too pronounced

(speltoid 174 and 298) or too small (speltoid 25_I and 34) a deviation from a monomeric segregation if considered from the phenotype of the homozygote (*cf.* the discussion about Fig. 76).

Another hypothesis which attempts to explain one source of def-type speltoids was based on a true gene mutation as the cause. With our present knowledge it is equally impossible to give a definite proof for or against, using induced or spontaneous material. Only one of the speltoids, *i. e.* 295, presented in Table 7, may possibly be considered as a gene mutation, the others not. Cytologically neither het nor hom speltoid 295 are to be distinguished from normal Scandia III. The chromosomes are apparently intact and the meiosis just as regular as the one found in the controls of Table 3:

	Metaphase I:		Tetrads:	
	total	2_I, % 1_{IV} or 1_{III}, %	total	abnormal, %
Het speltoid 295 ...	261	1,5 1,9	870	3,1
Hom » 295 ...	241	0,8 1,7	335	2,4

There are no genetic grounds either for ruling out a gene mutation as the cause. The homozygote is beardless and the heterozygote gives a very small deviation from a 1 : 2 : 1 segregation. This deviation unmistakably exists ($\chi^2=13{,}66$; $P=0{,}001$; heterogeneity, $\chi^2=9{,}49$; $P=0{,}48$), and for this reason a certain handicap of the speltoid-bearing pollen must be presumed. This change, however, may just as well depend on a gene mutation as on a minute deficiency, although the latter seems a little more likely. In the spring wheat material which was only genetically analysed, 9 cases at most out of 356, or 2,5 per cent, may possibly be included in the same category as that of speltoid 295.

From the foregoing it seems clear that complete identity as to the mutation mechanism exists between spontaneous and induced def-speltoids. Simple deficiency and deficiency-duplication definitely exist in the two materials, while chromosome substitution and gene mutation as the origin must occur very seldom, if they occur at all. The proposed name def-type for the whole of this group of speltoids thus seems quite representative.

Since the production of def-speltoids is mainly a process of deletion, there is, in addition to the mere qualitative one, another aspect of the identity between spontaneous and induced mutations. Do the breaks occur at random along the long arm of chromosome IX, or does this highly mutable chromosome have a weak point or points which react differently in the different materials tested? This question may be

answered by cytological analyses but would mean not only extensive work in getting representative data, but also great uncertainty. For these reasons this aspect of the identity was studied by purely genetic means.

In the introduction of this review of the speltoid problem, it was premised that the ratio-type was mainly regulated by the size of the IX-deletion. If that is true, the ratio of het speltoids to normals, determined by a sufficiently large segregation test, could be used as a measure of the variation in size of the deleted segment. If this really is the case, it can be proved by using dominant marker genes on the long IX-arm. We have already discussed two such genes, B_1 and Q, located at least 26 crossing-over units apart. Another useful factor is one regulating the spring wheat habit. This factor has been denoted S_K by ÅKERMAN (1943), and by ÅKERMAN and MAC KEY (1949) as being the single one regulating the spring habit of all Kolben wheats. The distance between this gene and B_1 and Q is not yet known but is under investigation. The sequence, however, given from the centromere outwards, is S_K—B_1—Q.

The Rival variety used in the experiments is just a monofactorial spring wheat of the genetic constitution $S_KS_KB_1B_1QQ$. In this variety, a small deficiency or point mutation including only the Q-factor, will thus give a hom speltoid which is a beardless spring wheat. If the B_1 is also included in the deletion, the homozygote will be a bearded spring wheat. With S_K also lost, the hom speltoid will appear to be a bearded winter wheat, provided that the deficiency is not so large that the hom speltoid will be lacking due to the non-functioning of the speltoid-bearing pollen. It is thus possible to group the present material as to the characteristics of the hom speltoid, and then study the distribution as to the different ratio classes within each group. Such an analysis is diagrammatically presented in Fig. 76 covering all X-ray and neutron induced def-speltoids from the 1948 experiment on Rival (total 273 cases; *cf.* Table 10).

A χ^2-analysis gives a significant heterogeneity between the four groups of def-speltoids ($\chi^2=119{,}64$; d. f.$=9$; $P<0{,}001$). A certain overlapping exists. This, however, is to be expected, and especially when considering the frequent positive or negative effect of the mechanism of deficiency-duplication. Nevertheless the tendency is quite clear. The more the phenotype of the hom def-speltoid is changed, the lower the functioning ability of the speltoid-bearing pollen, and thus the frequency of heterozygotes in the segregation test. This definitely means that the size of the deficiency in chromosome IX is mainly responsible for the ratio-type.

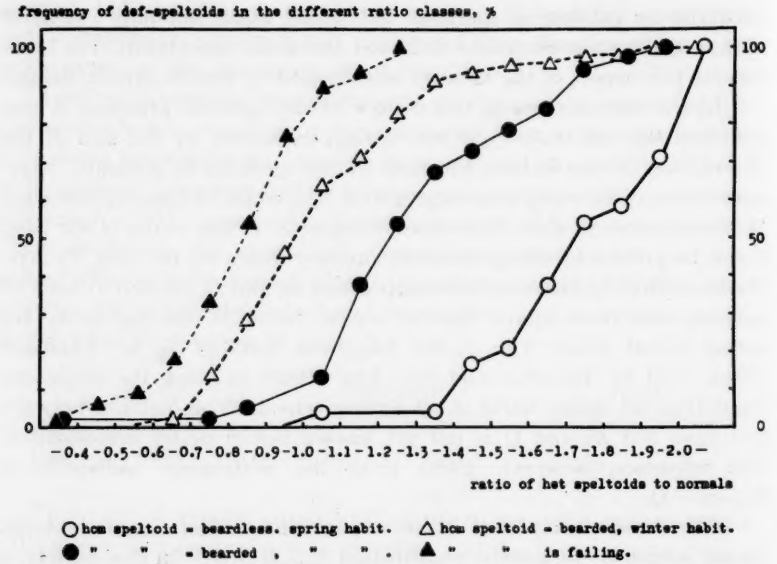

frequency of def-speltoids in the different ratio classes, %
100
50
0
-0.4 -0.5 -0.6 -0.7 -0.8 -0.9 -1.0 -1.1 -1.2 -1.3 -1.4 -1.5 -1.6 -1.7 -1.8 -1.9 -2.0-
ratio of het speltoids to normals
hom speltoid = beardless, spring habit.
hom speltoid = bearded, winter habit.
" " = bearded, " " .
" " is failing.

Fig. 76. Summation curves illustrating the distribution as to the ratio-types of induced def-speltoids of Rival spring wheat. The curves refer to types having a small, an appreciable, a large and a very large deficiency, respectively, in the long IX-arm. *Cf.* the text.

The frequency of heterozygotes in relation to normals may thus be used as means of finding out eventual differences between points of breakage or crossing-over in different material. In order to attack this problem as widely as possible, the def-speltoids considered are not only those originating from cross-material (KAJANUS, 1923), X-ray and neutron experiments, but also from a pure strain of Scandia III which was grown in the multiplication fields of the General Swedish Seed Company, Svalöf. The size of this material as well as the others are given in Table 10, where an indication is also given as to the exactness with which the individual ratio tests were made. A comparison between the def-speltoids of the four different origins as to their distribution in ratio classes is diagrammatically presented in Fig. 77. The conclusions that can be drawn from the diagram are the following:

(i) Def-speltoids of different origins show a very similar distribution as to ratio classes. An analysis of heterogeneity which was possible with a grouping into nine classes, does not show any significant difference ($\chi^2 = 25{,}59$; d. f. $= 24$; $P = 0{,}28$).

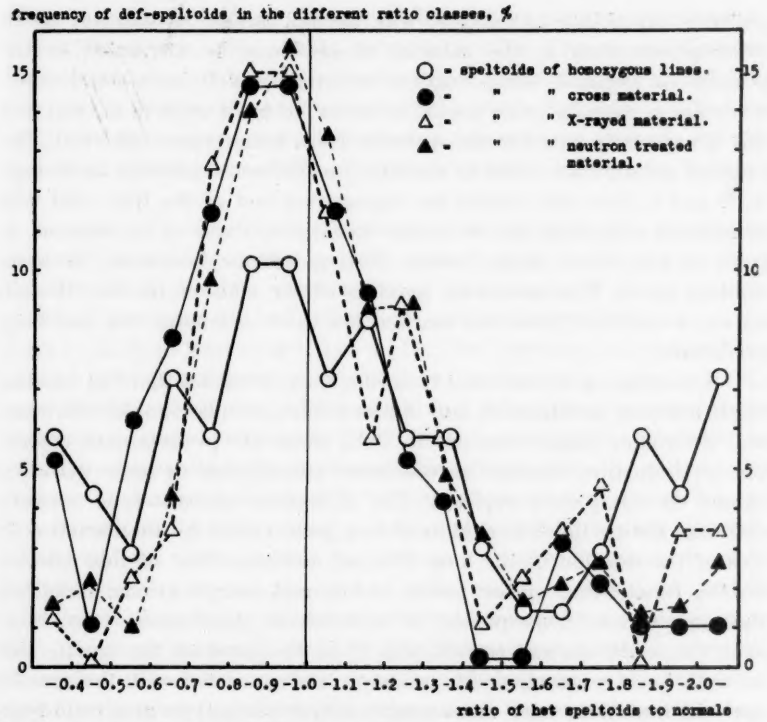

frequency of def-speltoids in the different ratio classes, %
○ - speltoids of homozygous lines.
● - " " cross material.
△ - " " X-rayed material.
▲ - " " neutron treated material.
15
10
5
0
−0.4 −0.5 −0.6 −0.7 −0.8 −0.9 −1.0 −1.1 −1.2 −1.3 −1.4 −1.5 −1.6 −1.7 −1.8 −1.9 −2.0−
ratio of het speltoids to normals

Fig. 77. The distribution in different ratio classes of def-speltoids of different origins. The vertical line indicates NILSSON-EHLE's limit between his type A and C.

(ii) NILSSON-EHLE's (1921) limit between his type A and C must be considered as less fortunately chosen. It nearly coincides with the peak of the distribution curve, which gives a further argument for the bulking proposed in the present paper.

(iii) No clear indication of a local concentration of breaks or crossing-overs at some point on the long IX-arm was revealed. There seems, however, to be a tendency to an accumulation of these events at the proximal and distal parts of the arm, which agrees with the general idea of the breakability of a chromosome along its length (*cf.* NORONHA WAGNER, 1950, and cited literature herein). The high number of cases with a normal : het speltoid ratio of about 1 : 1 does not mean that a certain size of the deficiency should prevail. Around this value all cases are gathered where a minimum chromosome loss deprives the

pollen of its ability to compete with normal pollen. A tendency to an overrepresentation in the extreme classes may be attributed to the positive or negative compensation involved in deficiency-duplication. A certain systematic error seems, however, to have crept in as well and this is especially true for the speltoids from homozygous material. The original attempt was only to classify the different speltoids as to type A, B and C. For that reason the segregation test of the first year was considered sufficient for very clear cases, *i. e.*, those of an extreme A, B or C ratio-type, while further testing became necessary in more dubious cases. The secondary maxima at the ends of the distribution curves would have been less marked if a more extensive test had been performed.

Summing up, it is evident from the above analyses that full identity exists between spontaneous and induced def-speltoids. Simple deficiency and deficiency-duplication are in both cases the predominant causes, but an induction through chromosome substitution or gene mutation cannot be completely rejected. The difference in ratio-type between different def-speltoids is explained to a great extent by the variation in size of the deletion in the long IX-arm, and the effect of this deletion on the functioning of the pollen. Additional complications, including the more serious consequences of a deficiency-duplication, may, however, cause the normal constitution to be favoured on the female side as well. Such rearrangements, more or less connected with the specific speltoid mutation, may considerably affect phenotype and ratio-type.

B. SPELTOID MUTATIONS OF MONOSOME-TYPE.

While the def-speltoids may vary considerably as to their genetic constitution and thus may be difficult to interpret in a correct and comprehensive manner, the monosome-speltoids show a more distinct mutation mechanism. The loss of the *Q*-factor and thus the appearance of the speltoid character occur through the loss of the whole *Q*-bearing chromosome, *i. e.*, normally the chromosome IX. The het speltoid is characterized by $2n=41$, and the hom speltoid which is always bearded by $2n=40$. WINGE (1924), in the first cytological investigation on speltoids, was already on the right track. He himself did not consider that he had found proof for such a mechanism in his own material, because he misinterpreted the significance of the univalent. Therefore, HUSKINS (1927, 1928) was the first one to give the concrete proof. This speltoid type, denoted by NILSSON-EHLE as type B and genetically characterized by an abnormally high frequency of heterozygotes, always

arises through monosomy. Repeated observations on spontaneous material (HÅKANSSON, 1930, 1931; MÜNTZING, 1930; PHIPPS and GURNEY, 1932; UCHIKAWA, 1936, 1941; SEARS, 1944; SANCHEZ-MONGE and MAC KEY, 1948; SMITH, HUSKINS and SANDER, 1949; JUNGFER, 1953) have confirmed this unanimously. Evidence that monosome-speltoids are also common in connection with mutagenic treatments is given in Tables 7 and 10.

In spite of a very distinct mechanism of mutation, the monosome-speltoids do not show one and the same ratio-type. As evident from Fig. 78, which gives the distribution in ratio classes of all monosome-speltoids accounted for in Table 10, the normal:het speltoid ratio varies from 1 : 3,5 to at least as much as 1 : 10. The frequency curve has, however, a pronounced peak at about 1 : 4, is markedly skew and falls successively towards the higher ratios. Just this picture of a successive transition makes LINDHARD's (1927) explanation of the variation in ratio-type impossible. He attributed the difference to the presence or absence of a special factor (*L*), which influenced the density of the ears as well as the functioning ability of the speltoid-bearing gametes. *LL*-plants with an especially lax ear were thought to produce active speltoid gametes and to give a 1 : 9 ratio, while *Ll* and *ll* resulted in 1 : 4 due to the sharply reduced activity of the speltoid gametes or to their complete inactivity, respectively. The present material, identical in origin, offers still another argument against this proposed interpretation by showing a complete absence of any correlation between head density and ratio-type.

It now seems clear that the lagging and exclusion of the univalent followed by an overproduction of (*n*-IX)-gametes may be influenced not only by external but also by heritable factors. This has been verified by checking the frequency of micronuclei in the pollen tetrads of different monosome-speltoids (HUSKINS, 1927, 1932, 1933, 1946; SANCHEZ-MONGE and MAC KEY, 1948; SMITH, HUSKINS and SANDER, 1949). Since the ratio-type is almost entirely determined by the interrelation between normal and speltoid-bearing egg cells (*cf.* Table 6), the analyses of the microsporogenesis are not conclusive. By crossing het speltoid × normal (or hom speltoid) it is, however, confirmed that the generalization is permitted.

No suggestions as to the factors influencing the behaviour of the univalent were made previously. Differences in the function of the centromere may be of certain importance. SEARS and CAMARA (1950, 1952) have shown that differences in the strength of the centromere

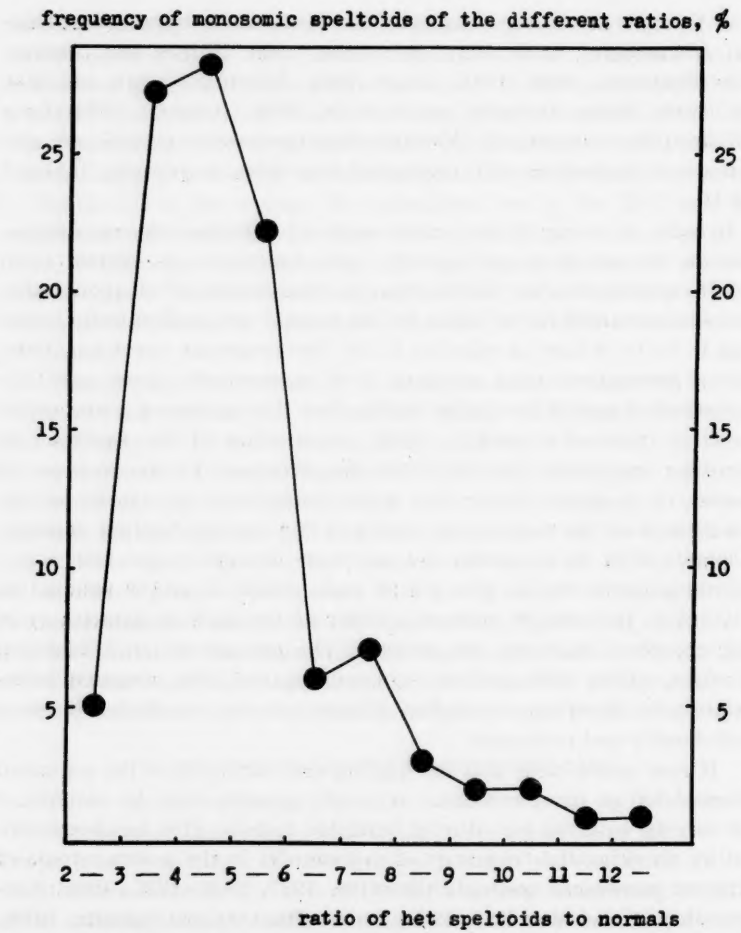

frequency of monosomic speltoids of the different ratios, %
25
20
15
10
5
2 — 3 — 4 — 5 — 6 — 7 — 8 — 9 — 10 — 11 — 12 —
ratio of het speltoids to normals

Fig. 78. The distribution of monosome-speltoids as to the different ratios of het speltoids to normals. The frequency curve is based on all monosome-speltoids accounted for in Table 10.

may occur in wheat, and LIMA-DE-FARIA (1953) has suggested a simple explanation for such differences. The pronounced divergency in the frequency of misdivided IX-univalents in the material analysed by SANCHEZ-MONGE and MAC KEY (1948) and SEARS (1952 a) indicates that qualitative differences also may exist in the centromere of chromosome

IX. Since the distribution on ratio classes of the present monosome-speltoids of spontaneous and induced origin shows very good conformity, it is, however, difficult to imagine this explanation as one of more general application. Everything indicates that deletions in the univalent, with or without segments translocated from other chromosomes, are responsible for a deviating behaviour. Large deficiencies, at least, seem to increase the tendency to exclusion, while added segments may have a reverse effect. Such additional complications do not presuppose an artificial induction, since misdivision may occur spontaneously as a process of deletion, and non-homologous crossing-over means a segment interchange at times of unequal size. Such a translocation may also happen to the *Q*-factor itself resulting in a chromosome other than the IX acting as the speltoid chromosome.

Even if the behaviour of the univalent seems to be mainly regulated by the univalent itself, extraneous factors may complicate the situation. A simultaneous induction of other mutations may lead to such complications. The ability of chromosome IX to influence the total frequency of chiasmata (*cf.* HUSKINS *et al.*, 1932, 1933 a and b, 1946) may perhaps be noticeable in this discussion as well.

The functioning ability of the (*n*-1)-gametes also influences the ratio-type. The compensative ability of the remaining germ plasm may certainly be varying, especially if the monosomy results from failure to pair in a deficiency-duplication (*cf.* Table 9). The environment must also be an important factor in this connection.

C. THE INTERRELATION OF DEFICIENCY- AND MONOSOME-SPELTOIDS OF DIFFERENT ORIGINS.

As evident from the foregoing, the def-speltoid is mainly produced by a break in the long arm of chromosome IX or by a crossing-over between this and another chromosome lacking the *Q*-factor. The monosome-speltoid is a result of non-pairing, lagging, and loss of the whole *Q*-bearing chromosome. Thus, the impulses are decidedly different and there are no reasons to presuppose that the interrelation of these distinctly separated types of speltoids should be identical, irrespective of their origin. Concerning the spontaneous speltoids, HUSKINS and SANDER (1949, p. 337) stated that »in wheat there is little difference in the frequency with which the three Series (A, B and C) have been found». This sweeping statement may be thought to correspond with the truth, but if an attempt is made to group the spontaneous material,

TABLE 10. *The distribution between deficiency- and monosome-speltoids in spontaneous and induced material.*

Origin	Observed No. of						Total	Mean No. of plants per progeny test
	beardless def-speltoids		bearded def-speltoids		monosome-speltoids			
	abs.	%	abs.	%	abs.	%		
Homozygous material, Scandia III	2	1,2	67	38,9	103	59,9	172	57 ± 3,8
Cross-material (KAJANUS, 1923)	2	2,0	94	92,1	6	5,9	102	221 ± 61,3
X-ray treated material, Rival	10	9,4	77	72,7	19	17,9	106	141 ± 9,0
Neutron treated material, Rival	13	5,2	173	69,2	64	25,6	250	112 ± 5,0

one will find greater divergences here than what may be produced by artificial means. In order to illustrate this condition, the induced def- and monosome-speltoids will be compared as to their relative occurrence, not only with speltoids from an established pure strain, but also with those reported by KAJANUS (1923) in his cross-material. The total analysis of the material comprising a little more than 75.000 progeny plants is computed in Table 10.

X-ray and neutron induced speltoids do not show any significant difference in the distribution to different types ($\chi^2=4{,}07$; d. f.$=2$; P$=0{,}13$). This is quite in accord with the earlier, though more roughly proved similarity in the relative ability of X-rays and neutrons to produce the different types of mutants (*cf.* p. 95; and for chlorophyll mutations in barley, MAC KEY, 1951). The tendency to a higher frequency of monosome-speltoids in N_2 may be due to the higher destructibility of the neutrons, as a severely affected chromosome may easier be lost. This agrees with the fact that the simultaneous appearance of both a def- and a monosome-speltoid in one head progeny occurred in 3,9 per cent (9 : 232) of N_2 but only in 1,0 per cent (1 : 104) of X_2.

If the induced X_2 and N_2 speltoids are compared with spontaneous speltoids from hom and het material, conformity will in no case be obtained as to the distribution in def- and monosome-type. The induced speltoids show too high a frequency of def-types if compared with the spontaneous speltoids of a pure strain ($\chi^2=67{,}97$; d. f.$=1$; P$<0{,}001$), while the reverse holds true in a comparison with speltoids obtained from crosses ($\chi^2=15{,}43$; d. f.$=1$; P$<0{,}001$). As a logical consequence of

these observations, the speltoids of the two spontaneous origins show a divergency still more significant ($\chi^2=77{,}94$; d. f.$=1$; $P<0{,}001$).

The distribution as to types of the speltoids originating from untreated homozygous material coincides fairly well with the meiotic abnormalities observed in such a material. One should expect an even still higher percentage of monosome-speltoids judging from Table 3, where the frequency of univalents *versus* fragments is presented. A certain discrepancy, however, is already expected due to the lower functioning of non-IX in comparison with def-IX gametes; further discrepancies are expected from the occurrence of def-dupl-speltoids as a consequence of non-homologous crossing-over and, finally, from the existence of somatic mutations.

It is interesting to note that the increased absolute frequency of speltoid mutations by mere crossing is mainly obtained by an additional production of def-speltoids. Since the increase by mutagenic treatments shows a similar tendency, there is a striking conformity in the acceleration of the mutation process in the two cases.

This apparent conformity, however, only holds good for the end results. The impulses can hardly be identical. The artificially induced speltoids of Table 10 were to a great extent already produced in somatic cells at the time of irradiation or immediately afterwards. This is proved by the high frequency of speltoid chimeras in the X_1 and N_1 generation. Considering the action of the mutagens, it is expected that def-type speltoids should prevail. Even the monosome-speltoids must be mainly considered as final consequences of all the chromosomal breaking expected after the applied doses.

The majority of the spontaneous def-speltoids cannot, however, be of somatic origin. They must have occurred during meiosis. Otherwise the difference in frequency and distribution as to the type of speltoids from homozygous and heterozygous material will be difficult to explain. A frequency of speltoid chimeras with no apparent differences in the two spontaneous materials further supports the idea that the additional occurrence of def-speltoids after crosses is meiotically conditioned.

As long as the individual chromosomes cannot be identified with certainty, it will be very difficult to give an absolute proof as to the cause of the spontaneous def-speltoids. Some indirect observations may, however, hint at the solution. A self-fragmentation of chromosome IX independent of external influences seems less likely when considering the sharp difference in the frequency of def-speltoids in homozygous

TABLE 11. *The frequency of chromatid bridges in first and second anaphase of some deficiency-duplications.*

Material	No. of late anaphases I			No. of late anaphases II	
	total	with bridge		total	with bridge, %
		%	+fragm., %		
Scandia III, control material	286	8,7	0,7	91	2,2
Hom def-dupl-speltoid 153	62	11,3	1,6	20	0,0
Hom def-dupl-speltoid 174	74	17,6	4,1	10	10,0
Hom def-dupl-compactoid 174	93	25,8	2,2	24	20,8
Tr. Spelta (SANCHEZ-MONGE, 1949)	152	15,3	0,0	113	3,5

and heterozygous material. The evidence of a random size of the def-segment (*cf.* Fig. 77) may also contradict such an idea. Misdivision, for example, must be of minor importance, though scarcely negligible. The absence of fragments in M I and early A I further rejects as an explanation a radiomimetic prophase breakage such as observed by WALTERS (1950) in the hybrid *Bromus Trinii*×*B. maritimus*. A less drastic and more acceptable explanation may be found in the existence of semi-homologous pairing in 6*x*-wheat. If crossing-over occurs, def-dupl-speltoids at least may be produced according to the above-mentioned scheme. There is, however, indication that even simple deficiencies occur from semi-homologous pairing. In order to discuss this possibility we have to go back to the bridges observed in A I and T I of the control material of Scandia III. In the review of this analysis given on pp. 84—86, no definite explanation could be presented as to the cause of these bridges. The meiotic characteristics of the def-dupl-mutations make it very probable, however, that these bridges, generally without fragments, are due to a delayed terminalization of semi-homologous crossing-overs. The fact is that these bridges are definitely increased in a def-dupl-mutant, where an increased pairing of chromosomes not identical along their length is the main feature (*cf.* Table 11 and Figs. 35—37, 42—47). Such an explanation is also consistent with the fact that similar bridge formations were found to be especially frequent in haploid and triploid material as well as in species crosses (*l. c.*, p. 84).

Since the number of bridges sharply decreases from anaphase to telophase, a great many of them will be resolved without fragmentation. Deficiency-duplications are, however, not excluded by this. Fragments

observed in later phases indicate breakage and deleted segments. This is already obvious in the control material (*cf.* Table 3) but is still more pronounced in def-dupl-mutants.

The occurrence of a delayed terminalization of chiasmata along non-homologous chromosome sections is subject to conflicting opinions. DARLINGTON (1936, and literature cited herein) is of the opinion that an interstitial crossing-over generally results in chromatid non-disjunction at the point of translocation. MARQUARDT (1948) and HAGBERG (1954) report, however, that normal terminalization occurs. From the present material, as well as from that of HAGBERG (*l. c.*), it seems likely that the discrepancy is only apparent. The truth seems to be intermediate. Terminalization along non-homologous sections certainly means an increased friction but not an insuperable one. Complications do not regularly occur but are relatively frequent.

The above discussion has dealt with chromosomes in general, but in order to explain the spontaneous occurrence of def-speltoids, the behaviour of chromosome IX must be regarded as being decisive. Conclusive data on this point are lacking. An insufficient analysis of the meiosis in hom nullisome-speltoids indicates, however, that at least a part of the T I bridges of normal *vulgare* plants include the chromosome IX. Its high mutability and indications of an inverted repeat in its long arm (SMITH, HUSKINS and SANDER, 1950) may be further reasons to suppose that a non-homologous crossing-over including chromosome IX will not always occur without complications.

If the explanation given above is accepted, the spontaneous occurrence of simple def- and def-dupl-speltoids is due less to an unstable point in the germ plasm than to the gene reduplication inherent for a polyploid. The more pronounced the homology between not completely identical chromosomes, the greater the chances of interpairing. Along with somatic mutating, the spontaneous occurrence of speltoids should mainly be caused by failing to pair, or by semi-homologous pairing of chromosome IX resulting in the monosome- and the def-type, respectively. These two primary causes are not only haphazard, but are also genetically regulated and this is especially true for the semi-homologous pairing. This condition admirably explains why the frequency of speltoids is found to vary from variety to variety and from cross to cross.

From the above it seems logical to expect a higher relative frequency of def-dupl-speltoids in spontaneous than in induced material. Considering the high frequency of translocations with the high doses

applied, the rather frequent chromatid non-disjunction following semi-homologous crossing-over and, finally, the tendency of a deficiency-duplication to change into a simple deficiency, the relation to type should not necessarily be markedly different in the two cases. Processes of simple segment loss, *e. g.* misdivision, must further be considered to occur in spontaneous material as well, and semi-homologous pairing may be stimulated by the meiotic disturbance that generally follows a mutagenic treatment.

2. DENSE-EARED MUTATIONS.

In contrast to the speltoids, dense-eared mutations cannot be attributed to the change of just one single gene. Crosses between representatives of this group, which is fairly homogeneous phenotypically, definitely reveal several loci to be responsible. This is also consistent with the observation of my colleagues HAGBERG and NYBOM that the dense-eared *erectoides* mutations in the diploid barley may occur in at least sixteen different loci (*cf.* HAGBERG, 1954). The complexity of the dense-eared mutations thus makes them difficult to analyse. In the present study on induced mutations in wheat, the analysis of this group thus far is only completed as to the progeny test and the morphological description. Table 12 gives the main characteristics of some dense-eared mutations of X-rayed Scandia III, since the more detailed analyses are being concentrated on this material. The dense-eared mutations show an apparent correlation between plant height and head density, the latter measured by the number of spikelets per 100 mm of rachis length (D-value). Scandia III, normal, has $D = 26{,}9 \pm 0{,}087$; $n = 400$. The different mutations reveal different ability as to phenotypical expression in the heterozygote condition. The ratio-type of the dense-eared mutations generally equals that of the def-speltoids, *i. e.*, a deficit of heterozygotes and especially of homozygotes, indicating a lower functioning of the gametes bearing the dense-eared mutation.

In the first place the detailed studies are an attempt to establish if duplications are important as a source of dense-eared mutations in 6*x*-wheat. Here the cytological analyses are also verified by genetical methods. Thus, duplications of the *Q*-factor resulting in the so-called compactoids are checked by outcrossing with a hom speltoid. In conformity with Fig. 53, the F_1 will be a restoration of the original mother type, if the dense-eared mutation is a true compactoid. If not, the F_1 becomes a het speltoid, generally with the laxness dominating over the density. A similar balance, and thus a possible deficiency *versus* du-

plication, has also been obtained from crosses between dense- and lax-eared mutations of the same X_2 line. Since the dense-eared mutations often showed very low fertility, the analyses proved to be difficult to carry through and are thus not completed. They indicate, however, that about half of the dense-eared mutations in wheat are duplications.

Due to their close relation to the speltoid problem, the compactoids were more thoroughly studied than the other types of dense-eared mutations. The compactoid is characterized by an overdose of *Q*-factors. It is important to establish this definition, even if the term compactoid, proposed by KAJANUS (1927) as a substitution for NILSSON-EHLE's (1920) confusing term Compactum, is open to criticism. This denotation, as well as its predecessor, hints at the similarity of the mutation with *Triticum compactum* HOST. Superficially, they have also several features in common, such as a dense club ear, rounded loose glumes with faintly marked nervature and a thick short straw. The density of the head is, however, not as pronounced and the ear is somewhat more square-headed in the compactoid. Further, this mutation has the glume very faintly keeled and the characteristic crookedness of the *compactum* straw just under the head is either lacking here or less marked. From a purely genetical point of view, the two types have nothing to do with each other. This is obvious from a cross with a speltoid whose phenotype in one case will be counterbalanced, and not in the other (Fig. 84). The cross compactoid×*Tr. compactum* with dominance for the latter shows a typical dimeric segregation:

Compactoid 174×*Tr. compactum*, F_2:

	normal type	subcompactoid	compactoid	het *compactum*	hom *compactum*	Total
Observed	10	23	7	75	44	=159
Expected, rel.	1 :	2 :	1 :	8 :	4	
» , abs.	9,94	19,87	9,94	79,50	39,75	

$\chi^2=2{,}07$; d. f.=4; P=0,73.

UNRAU (1950) and UNRAU, SMITH and MC GINNIS (1950) have also shown that the head density of *Tr. compactum* is regulated by chromosome XX, and that of the compactoid by chromosome IX. In spite of this non-identity, the term compactoid will be retained here for the *Q*-regulated dense-eared mutations, as this term has been generally accepted, and also since no type of mutation more closely related to *Tr. compactum* has as yet been obtained. It seems further advisable to follow the old nomenclature of NILSSON-EHLE by denoting the hetero-

TABLE 12. *Analysis of induced dense-eared mutations, Scandia III, 20.000 r.*

X_2-line 1945—959—	Difference: dense-eared — normal type				Segregation in offspring of the het dense-eared mutation:							Mutation mechanism
	het		hom		absolute values:						ratio-type	
	plant height cm	ear density	plant height cm	ear density	normal type	het dense-eared	hom dense-eared	het. spelt.	sub-normal	Total	n : het : hom	
1	— 10	+ 2,3	— 17	+ 7,9	90	156	66	—	—	312	1 : 1,7 : 0,73	point mutation
14	— 3	+ 4,5	— 10	+ 12,0	20	46	23	—	—	89	1 : 2,3 : 1,15	
18	— 14	+ 7,3	— 70	+ 13,5	395	306	9	—	—	710	1 : 0,8 : 0,02	
22_I	— 19	+ 9,6	— 51	+ 10,8	350	302	30	—	—	682	1 : 0,9 : 0,09	
22_{II}	— 9	+ 1,6	— 18	+ 8,3	17	48	20	—	—	85	1 : 2,8 : 1,18	
25_I	— 18	+ 7,3	— 44	+ 10,1	357	484	127	13	—	981	1 : 1,4 : 0,36	tri-IX
25_{II}	— 22	+ 7,2	— 52	+ 9,8	132	136	12	80	13	373	1 : 1,0 : 0,09	iso-IX
34	— 10	+ 4,2	— 20	+ 7,2	36	63	15	—	—	114	1 : 1,8 : 0,42	
61_I	— 3	+ 1,8	— 8	+ 5,8	78	142	76	4	—	300	1 : 1,8 : 0,97	point mutation
61_{II}	— 14	+ 3,2	— 25	+ 9,8	72	117	44	—	—	233	1 : 1,6 : 0,61	
76	— 2	+ 2,6	— 6	+ 8,2	46	128	34	—	—	208	1 : 2,8 : 0,74	
80	— 22	+ 5,9	— 58	+ 13,1	419	367	6	—	—	792	1 : 0,9 : 0,01	tri-IX
93	— 19	+ 10,9	— 77	+ 12,2	321	276	4	—	—	601	1 : 0,9 : 0,01	
136	— 14	+ 3,5	— 35	+ 10,7	269	305	31	11	—	616	1 : 1,1 : 0,12	

					321	276	4	—	—	601	1 : 0,9 : 0,01	
136	−14	+ 3,5	−35	+ 10,7	269	305	31	11	—	616	1 : 1,1 : 0,12	
139	−21	+ 6,5	−51	+ 11,3	321	190	7	—	—	518	1 : 0,6 : 0,02	
141	−21	+ 9,1	−58	+ 13,1	136	125	17	4	—	282	1 : 0,9 : 0,13	
149	−26	+ 8,1	−51	+ 9,8	322	312	30	—	—	664	1 : 1,0 : 0,09	tri-IX
156	−21	+ 5,7	−63	+ 8,4	489	307	23	1	—	820	1 : 0,6 : 0,05	
174	−20	+ 8,7	−40	+ 12,6	182	277	128	—	—	587	1 : 1,5 : 0,70	def-dupl-IX
184	−20	+ 9,7	−42	+ 16,5	270	289	76	—	—	635	1 : 1,1 : 0,28	
209	−11	+ 8,3	−30	+ 15,0	371	376	91	—	—	838	1 : 1,0 : 0,25	
248	−24	+ 7,1	−43	+ 11,9	162	194	25	—	—	381	1 : 1,2 : 0,15	
279	−18	+ 7,3	—	—	197	73	—	—	—	270	1 : 0,4 : 0,00	
286	−11	+ 4,0	−20	+ 10,6	195	150	1	—	—	346	1 : 0,8 : 0,01	
293	− 8	+ 0,6	−14	+ 5,6	33	60	45	—	—	138	1 : 1,8 : 1,36	
329	−22	+ 8,5	−63	+ 11,3	298	195	2	—	—	495	1 : 0,7 : 0,01	
351	− 5	+ 2,0	−14	+ 10,7	143	286	119	—	—	548	1 : 2,0 : 0,83	
373	−22	+12,2	−56	+ 12,9	212	240	45	92	24	613	1 : 1,1 : 0,21	iso-IX
383	−31	+ 4,0	−85	+ 8,3	213	108	3	—	—	324	1 : 0,5 : 0,01	
390	−19	+ 3,2	−52	+ 7,9	281	263	58	—	—	602	1 : 0,9 : 0,21	
402	−16	+ 7,5	−41	+ 14,1	296	382	167	—	—	845	1 : 1,3 : 0,56	def-dupl-IX
407	− 6	+ 3,3	−15	+ 9,2	41	51	28	—	—	120	1 : 1,2 : 0,68	
M_{diff}	−16	+ 5,9	−40	+ 10,6	6764	6754	1362	205	37	15122		
$m_d\pm$	1,30	0,52	3,90	0,47								

zygote by the term subcompactoid and the homozygote by compactoid only. The prefix »homozygote» is less fortunate in this special case, since the compactoid seldom gives a constant progeny due to its characteristic genetic constitution.

The subcompactoid, more seldom the compactoid directly, generally occurs in connection with, or as a secondary product of, het speltoids (NILSSON-EHLE, 1920, 1921; VESTERGAARD, 1921; LINDHARD, 1922; ÅKERMAN, 1923; ISHIKAWA, 1934; VASILJEV and KAMENIK, 1935; KATTERMANN, 1937; UCHIKAWA, 1937, 1938, 1939, 1941, 1942 b, 1943 a and b; SEARS, 1944, 1952 a; ÅKERMAN and MAC KEY, 1948; LI, HSIA and LEE, 1948; SMITH, HUSKINS and SANDER, 1949, 1950; JUNGFER, 1953). No definite account is available as to whether the mutation may occur independent of speltoids. BOSHNAKIAN (1922), LINDHARD (*l. c.*) and DUMON (1931), however, report the appearance of apparently true compactoids even independent of speltoids. Dense-eared or squareheaded mutations, probably including true compactoids, have been reported in experiments with X-rays (DELAUNAY, 1930, 1931, 1932, 1934; SAPEHIN, 1935; FRÖIER, 1946) and with radiophosphorus (ARNASON, PERSON and NAYLOR, 1952), or based on seed which had been stored over a long period (SCHKWARNIKOW, 1937, 1939).

All previous interpretations of the origin and genetical constitution of compactoids are based on spontaneous cases segregated from the monosome-speltoid or its derivatives. NILSSON-EHLE himself (1920) did not at first consider his identified aberrant as a new mutation. He interpreted it as a recombination possible through the complex process of the primary speltoid mutation. LINDHARD (1923) was the first one to consider the compactoid to be a new chromosomal rearrangement, a new mutation, although he erroneously, and contrary to his own data of 1922, asserted that this process was only possible in a mono-IX genotype. A further step in the study of the compactoid was made by WINGE (1924) from cytological investigations, when he considered the mutation to be caused by an overdose of chromosome IX (his chromosome C). However, later on his interpretation proved to be too schematic, as he did not, nor could he, anticipate all the possibilities by which the *Q*-factor could be duplicated. He got off on the wrong track with his suggestion that the compactoid phenotype would occur, if that certain chromosome was lost which was homoeologous to and counterbalancing the chromosome IX. This way of reasoning was also accepted by UCHIKAWA (1939, 1943 a) for his »type II dwarf-compactoid». SEARS' (1944) nulli-V and nulli-XVIII are definite disprovals, since the heads of these

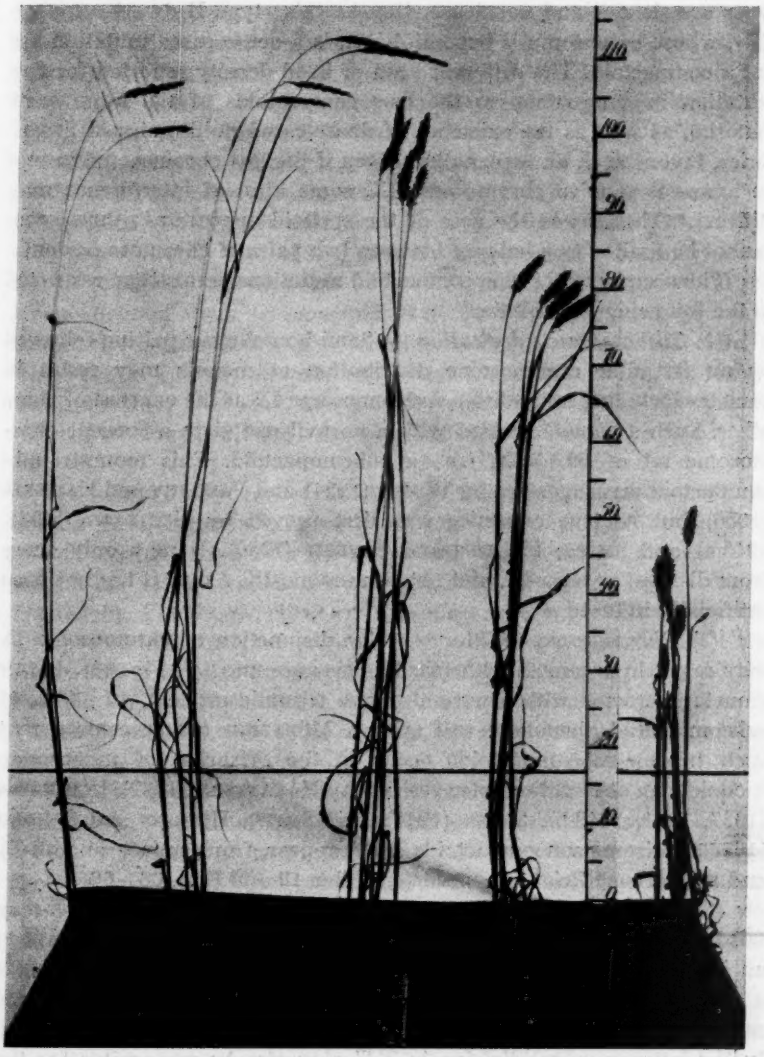


Fig. 79. The effect of chromosome IX with different doses on the phenotype of 6x-wheat, illustrated by X-ray mutants of Scandia III. — From left to right: nulli-IX (hom speltoid), mono-IX (het speltoid), di-IX (normal type), tri-IX (subcompactoid), and tetra-IX (compactoid).

types are slender and not dense. UCHIKAWA's »type II dwarf-compactoid», whose monosomy is beyond doubt, is a dense-eared mutation but not a compactoid. The different kind of head density reported for this mutation in comparison to the true compactoids of the same basic material, as well as the existence of dense-eared nullisomics in SEARS' series, favour such an explanation. Even if the lost chromosome cannot be homoeologous to chromosome IX, some kind of interference may still exist. Here, as in the case of the speltoid appearance, the system cannot be limited to a balance between two pairs of chromosomes only.

The occurrence of true compactoid mutations seems thus restricted to the following possibilities:

(1) *Chromosome substitution.* — Semi-homologous pairing followed by an irregular chromosome distribution at meiosis may result in gametes including an additional chromosome IX at the expense of some other. Such a gamete crossed with a normal one gives a somatic chromosome set of 39+3 IX, *i. e.*, a subcompactoid. This mono-tri-subcompactoid was suggested by WINGE (1924) and VASILJEV and KAMENIK (1935) but its true existence was first proved by UCHIKAWA (1938, 1943 a) and SMITH, HUSKINS and SANDER (1950). It may only occur from di-, tri-, or tetra-IX, and not from mono-IX. As yet it has not been artificially induced.

(2) *Chromosome addition.* — Non-disjunction of chromosome IX may result in a gamete with two IX chromosomes (*cf.* Fig. 63). If this gamete is crossed with a normal one, a trisomic product, 40+3 IX, of subcompactoid phenotype will appear. MU[illegible]ING (1930) showed that such trisome-subcompactoids occur in the offspring of monosome-speltoids, an observation later verified by HÅKANSSON (1932), UCHIKAWA (1937, 1941, 1942 b), SEARS (1944), and SMITH, HUSKINS and SANDER (1950). Trisome-subcompactoids may appear from mono- and di-IX and are here artificially induced (*cf.* Table 12 and Figs. 66—68).

(3) *Isochromosome formation.* — An unpaired chromosome IX may misdivide in T I (*cf.* p. 86 and Figs. 12, 64 and 65) and give rise to an isochromosome with the long arm and the *Q*-factor duplicated as a consequence. This isochromosome may occur directly by transversal misdivision or by non-disjunction of a telo-IX in a subsequent division. In the latter case, misdivision in T II may also be responsible for the production of these iso-subcompactoids of the constitution 40+IX+iso-IX. The correct interpretation of this type of mutation proceeded step by step. HÅKANSSON (1932), and later KATTERMANN (1937), called attention to a certain identity between the two arms of the iso-IX. SANCHEZ-

MONGE and MAC KEY (1948) linked this observation with the phenomena of misdivision and isochromosomes, complications whose importance in this matter was verified not only by LI, HSIA and LEE (1948) and SMITH, HUSKINS and SANDER (1950) but also by SEARS (1952 a) who made the final revision.

The iso-subcompactoid is a frequent secondary mutation in monosome-speltoids and was earlier observed in this connection only. The existence of this type of mutant in X_2 and N_2 shows, however, that it may occur directly and in this case not only associated with speltoids (*cf.* compactoids 25_{II} of Table 12 and Figs. 69—72) but also entirely independent of them (compactoid 373). Its frequency in spontaneous material is regulated by a different tendency of the IX-univalent to misdivide, in T I varying between at least 1,7 per cent (SANCHEZ-MONGE and MAC KEY, *l. c.*) and 43,5 per cent (SEARS, *l. c.*). In the present material of Scandia III the corresponding value proved to be 6,1 per cent (22 : 360).

(4) *Simple duplication.* — A duplication in chromosome IX including the *Q*-factor will result in a compactoid. Such a dupl-subcompactoid, characterized by a short terminal duplication, was reported by HÅKANSSON (1932) and a similar type with the entire long arm duplicated by UCHIKAWA (1942 b). The dupl-subcompactoid type might be expected in connection with the earlier discussed bridges in T I and T II, where a special breakage pattern may produce an inverted duplication (*cf.* FRANKEL, 1949). Though not yet definitely proved to be artificially induced, they certainly will be rather frequent in irradiated material.

(5) *Deficiency-duplication.* — A mutant with the *Q*-factor represented in two different pairs of the chromosomes may occur as a secondary product of non-homologous crossing-over or translocation. This type of *Q*-duplication was not known previously but its existence was definitely proved in connection with the above analysis of the analogous speltoid (*cf.* pp. 111—116, Tables 8 and 12, Figs. 29—37, 39). The def-dupl-subcompactoid will be mainly expected to occur from the normal type directly.

It must be emphasized that the above-mentioned origins of compactoids are to be considered as prototypes only. The ability of chromosome IX to pair at times with other chromosomes, and the complications following misdivisions and non-disjunction, offer several possibilities for slight varations. The origin, stability, transmissibility and genetical effect of these IX-derivatives and non-IX chromosomes

TABLE 13. *Different compactoid mutations, their constitution, segregation and denomination.*

$2n =$	No. of *Q*-factors	Proposed term:	Genotypic constitution: 40 +	Main types in the offspring and their frequency: n : subc : comp : subn : het s : hom s	Terms used previously:
			I. *Chromosome substitution:*		
42	3	mono-tri-subcompactoid	3 IX— non-IX	$1 : < 2 : < 1$	ACC/ABC-Typus (W), type I het dwarf-compactum (U), type I dwarf-compactoid het (U).
42	4	nulli-tetra-compactoid	4 IX—2 non-IX	$- : < 0{,}1 : 1$	ACC/ACC-Typus (W), type I hom dwarf-compactum (U), type I dwarf-compactoid hom (U).
			II. *Chromosome addition:*		
43	3	trisome-subcompactoid	3 IX	$1 : \leqq 1 : \leqq 0{,}1 : - : \leqq 0{,}1$	type I subcompactum (U), type I compactoid het (U), type II subcompactoid (S).
44	4	tetrasome-compactoid	4 IX	$< 0{,}1 : < 0{,}3 : 1$	type I short-compactum (U), type I compactoid hom (U), type II compactoid (S).
43	4	di-iso-subcompactoid	2 IX + iso-IX	$1 : \leqq 1 : \leqq 0{,}1 : - : \leqq 0{,}1$	type III subcompactoid (S).
44	6	di-iso-compactoid	2 IX + 2 iso-IX	$- : \geqq 1 : 1$	type III compactoid (S).
			III. *Isochromosome formation:*		
					(co+no)-subcompactum (H), type I

					type III compactoid (S).

III. *Isochromosome formation:*

42	3	iso-subcompactoid	IX + iso-IX	$1 : \leqq 1 : \leqq 0{,}1 : \leqq 0{,}6$	(co+no)-subcompactum (H), type I subcompactoid (S).
42	4	iso-compactoid	2 iso-IX	$- : < 1 : 1 : \cong 1$	type I compactoid (S).
41	2	iso-subnormal	iso-IX	$- : < 0{,}4 : < 0{,}3 : 1 : \leqq 1 : < 0{,}2$	
42	3	telo-iso-subcompactoid	telo-IX + iso-IX	$- : \geqq 1 : < 0{,}5 : 1 : < 1$	
42	2	het telo-subnormal	IX + telo-IX	$1 : \leqq 0{,}1 : - : < 1{,}5 : \leqq 0{,}1$	het CS_2-normal (U).
42	2	hom telo-subnormal	2 telo-IX	$- : \leqq 0{,}1 : - : 1 : \leqq 0{,}1$	hom CS_2-normal (U).

IV. *Simple duplication:*

42	3	dupl-subcompactoid	IX + *QQ*-IX	$1 : \leqq 2 : \leqq 1$ ($: < 0{,}1 : < 0{,}6$)	type II subcompactum (U), type II compactoid het (U).
42	4	dupl-compactoid	2 *QQ*-IX	$- : < 0{,}1 : 1$ or constant	type II short-compactum (U), type II compactoid hom (U).
41	2	dupl-subnormal	*QQ*-IX	$- : < 0{,}4 : - : 1 : < 0{,}1$	C′-normal (U).

V. *Deficiency-duplication:*

42	3	def-dupl-subcompactoid	2 IX + *Q*-segm.	$1 : < 2 : < 1 : < 0{,}1$	
42	4	def-dupl-compactoid	2 IX + 2 *Q*-segm.	$- : < 0{,}2 : 1$	
41	2	def-dupl-subnormal	IX + *Q*-segm.		

W=Winge (1924); U=Uchikawa (1942 b, 1943 a and b); S=Smith, Huskins and Sander (1950); H=Håkansson (1932).

were studied by HUSKINS (1946), LI, HSIA and LEE (1948), SMITH, HUSKINS and SANDER (1950), SANCHEZ-MONGE (1951), and SEARS (1952 a and b).

The different basic types and known variants of subcompactoids and compactoids have been computed in Table 13, where chromosome number, number of *Q*-factors, the proposed denotations, genotypic constitution, approximate ratio-type and earlier terms are presented. Speltoids, *i. e.*, a deficit of *Q*-factors, occur in the offspring with a frequency that fairly well reflects the ability of the different IX-derivatives to be transmitted. In certain cases, a new type is segregated which has the normal number of two *Q*-factors but nevertheless shows departure from type. It has a normal head but is generally somewhat shorter in straw and not so vigorous. These so-called subnormals may be phenotypically similar to each other but are by no means genetically alike, since they have different origins. The different subnormal types are accounted for in Table 13. Contrary to some trends, the term subnormal is here restricted to those rearrangements where only chromosome IX or its derivatives are involved.

Even if compactoid mutations of spontaneous and induced origin show a good cytogenetic conformity as in the case of the speltoids, there are also reasons here to suspect differences in the relative commonness of the different types. It is probable, though not proved, that trisome- and iso-subcompactoids are comparatively more common in pure strains, while the other types would be more likely to appear in cross-material or in connection with mutagenic treatments.

3. LAX-EARED MUTATIONS.

A mutation was registered as a lax-eared off-type if its progeny proved to have an ear density at least one D-unit below type (determined on ten ears each in the progeny of the heterozygote). Since the lax-eared mutations show a continuous deviation from type, such a requirement for a distinct departure undoubtedly underestimates the actual frequency. Along with the distinct speltoids, the lax-eared mutant group of Table 4 is nevertheless the largest one. With heavier doses of X-rays or neutrons, the frequency may exceed 30—40 per cent and about one third of the total number of mutations per dose are generally of the lax-eared non-speltoid type. This preponderant position coincides very well with the fact that lax-eared mutation and the speltoid are the most common aberrants in spontaneous material suitable enough for a distinct registration of ear laxity. Thus there is again a parallelism

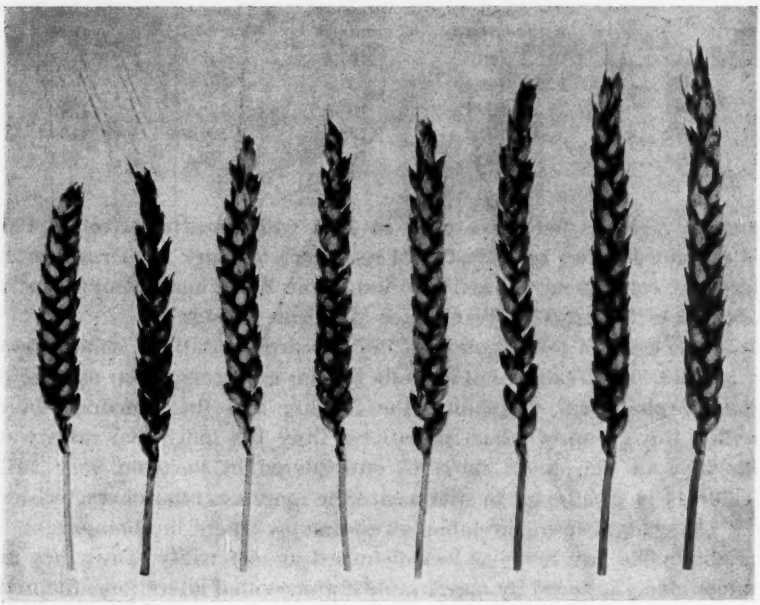


Fig. 80. To the left a head of Scandia III, normal type. Other heads represent different lax-eared X-mutants induced in this variety.

between spontaneous and induced production of observable mutations in 6*x*-wheat. A more conclusive proof would be very time-consuming for this special case, since the high frequency is shown to be more a matter of polymery than of one or few highly mutable loci. This is evident not only from the wide morphological variation within the group (*cf.* Fig. 80 and Table 14) but also from direct intercrosses. In addition, the cytological background of the mutations varies considerably. So far, meiotic analyses have revealed that point mutations, visible deficiencies, deficiency-duplications, and monosomy occur.

From a practical point of view, the lax-eared mutations are seldom of greater interest, an experience verified in Scandia III for both spontaneous and induced types. Some exceptions occur, however, which is evident from the results of the yield tests of three interesting X-mutants presented on p. 144. The grain yield is given in relative values.

The straw of the mutants is not so stiff as that of the mother line and the specific yielding capacity is not increased in the mutants 217

Year	Scandia III, standard	Lax-eared mut. 128	Lax-eared mut. 217	Lax-eared mut. 281
1949	100,0	107,2	—	—
1950	100,0	92,2	—	—
1951	100,0	103,4	128,3	124,4
1952	100,0	108,4	104,4	110,4
1953	100,0	103,0	89,3	97,8

and 281. The higher grain yield in 1951 with an after effect in 1952 is explained by an improved field resistance to black stem rust, an interesting consequence of artificial induction. For a more comprehensive account of the practical results, see MAC KEY (1954 b).

The detailed investigation of the lax-eared mutations, concentrated on the 20.000 *r* treatment of Scandia III, has only gone so far as to show the morphological variability, the fertility and the genetic stability within this group of wheat mutations. Only 108 individual cases were included in the study, since 43 outwintered in the cold year 1947. Table 14 is an attempt to summarize the more essential characteristics.

As evident from the table, all mutations where the homozygote is unobtainable, are more or less disturbed as to fertility. Thus they are undoubtedly induced by appreciable chromosomal alterations, the main part of which probably are deletions. A similar drasticity of the induced mutation seems also to be found in nearly half of the lax-eared off-types, where the homozygote could be satisfactorily fixed. It is thus evident that the majority of the lax-eared mutations in wheat are induced by chromosomal alterations. The existence of fertile and well established types makes it very probable, however, that point mutations including true gene mutations may be represented as well.

A comparison between the internodal length of the ear and the straw shows a certain correlation. These characteristics are represented in Table 14 by the ear density measured as the number of spikelets per 100 mm length of the rachis and by the height of the plants (the first culm here invariably with 5 nodes), respectively. The correlation, however, is not strikingly high within all the lax-eared mutations examined, $r = -0{,}249$ ($P < 0{,}01$). It is disturbed by more severe chromosomal rearrangements which is evident from the increased correlation ($r = -0{,}433$; $P < 0{,}01$) when only the 47 constant and fertile mutations are considered.

69 out of 108 progeny-tested lax-eared mutations, or nearly two thirds, must be classified as mutations in straw height as well. 30 mutations, including 19 fertile ones, have a longer straw than that of

TABLE 14. *Analysis of induced lax-eared mutations, Scandia III, 20.000 r.*

		Homozygote of the mutation	Ear density (= D): – 20,0		– 21,0		– 22,0		– 23,0		– 24,0		– 25,0		–		Total	
			fertile	semi-fertile	fertile	semi-fertile	fertile	semi-fertile	fertile	semi-fertile	fertile	semi-fertile	fertile	semi-fertile	fertile	semi-fertile	fertile	semi-fertile
Long-strawed mutation	Plant height: 120 cm (AII)	unobtainable	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
		obtainable	1	1	2	—	—	—	2	—	—	—	—	—	—	—	5	1
	115 cm	unobtainable	—	—	—	—	—	—	—	2	—	1	—	—	—	—	—	3
		obtainable	1	—	3	2	2	2	1	2	4	—	2	1	1	—	14	7
Normal plant height	110 cm	unobtainable	—	—	—	—	—	1	—	1	—	3	—	1	—	1	—	7
		obtainable	—	—	3	—	2	—	3	1	2	3	7	2	6	3	23	9
Short-strawed mutation	105 cm	unobtainable	—	—	—	—	—	2	—	2	—	2	—	1	—	1	—	8
		obtainable	—	1	—	—	—	—	1	1	1	4	—	2	—	—	2	8
	100 cm	unobtainable	—	—	—	—	—	—	—	1	—	—	—	2	—	1	—	4
		obtainable	—	1	—	—	—	—	1	—	1	1	—	—	1	2	3	4
	95 cm	unobtainable	—	—	—	1	—	—	—	—	—	1	—	2	—	—	—	4
		obtainable	—	—	—	1	—	—	—	1	—	1	—	1	—	—	—	4
	90 cm (VII)	unobtainable	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	1
		obtainable	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	1
Total		unobtainable	—	—	—	1	—	3	—	6	—	8	—	6	—	3	—	27
		obtainable	2	3	8	3	4	2	8	5	8	9	9	7	8	5	47	34

the mother line, while 39, including only 5 fertile types, have a shorter straw. The phenotype with a lax ear and a short straw is evidently an effect of appreciable chromosomal rearrangements and is thus hardly to be considered as due to monogenic pleiotropy.

The same interpretation seems to be true when considering other characteristics added to the laxness of the ears. Such additional deviations from type are most common in the non-fertile group, where more extensive changes than mere point mutations may be presumed to have occurred. Besides a pronounced variation in shape and looseness of the glumes, there were thus among the 108 lax-eared mutations:

23 with a reduced wax cover in the head region (including 4 fertile),
8 with a reduced wax cover over the whole plant (3 fertile),
6 with an abnormally pronounced wax cover (2 fertile),
15 with a heritable pseudo black chaff (2 fertile),
2 with bearded lemmas (2 fertile),
5 with delayed ripening (1 fertile),
5 with earlier ripening (0 fertile),
2 with spring wheat habit (0 fertile).

The transition from winter to spring habit is especially remarkable. These mutations were identified by spring sowing, a routine test made in order to check possibilities of outcrosses in the mutation material. Four lines segregated plants able to shoot even if they were decidedly later than even a French alternate wheat. One of the off-types definitely has proved to be an outcross, one is still doubtful, while two showed to be true mutations.

For the discussion on the evolutionary interrelation of the two types of growing habit, it is interesting to state from the present investigation that spring wheat can mutate to winter wheat (*cf.* p. 121), and *vice versa*. This is all the more interesting since there are divergent opinions as to which of the types is to be considered as the progenitor (*cf.* VAVILOV and KOUZNETSOVA, 1921). A cytogenetic study of the induced mutations may perhaps throw some light on this question of differentiation. Such analyses are in progress.

4. BEARDED MUTATIONS.

Following the symbolization of WATKINS and ELLERTON (1940) for the awn factors, the two varieties Scandia III and Rival are classified as $B_1B_1b_2b_2$-types, *i. e.*, they are not strictly awnless but »tipped 1» Since a direct mutation of the recessive to the dominant allelomorph is rare,

the genic constitution of the two varieties used automatically associates all observable changes in awn appearance with the B_1-gene of chromosome IX. All bearded mutants of Scandia III, including all bearded speltoids (see above), were also proved to be mutations including the B_1-locus. The same holds true for the spontaneous bearded mutation which occurred in the control material of Scandia III as well as for the bearded mutations obtained in the Rival experiments. In a beardless wheat of the European type, the bearded mutations thus are a very homogeneous group from a genic point of view.

This does not, however, mean that the mutation processes are always identical. As shown above, the bearded speltoids generally occur through a loss in the long IX-arm of a terminal segment at least 30 crossing-over units in length. It is hardly likely that a true gene mutation will produce the awns here. Though cytological proofs are still lacking, it seems very likely that deletions are more common than gene mutations within the induced bearded mutations of non-speltoid appearance as well. In this case, however, the deletion will be interstitial and not as wide. A variation in size is indicated by the fact that bearded X- and N-mutants of Scandia III and Rival show a variation in grain yield from 81,5 to 102,9 per cent if compared to the mother line. It is somewhat discouraging to note that none of the Scandia III and only three of the Rival mutants were able to retain the yielding capacity of the mother, while the single spontaneous mutant, indicating a true gene mutation, gave the following figures:

Year	Relative grain yield: Scandia III, mother line	Relative grain yield: Bearded spont. mut.	1000-grain-weight, gms.: Scandia III, mother line	1000-grain-weight, gms.: Bearded spont. mut.
1949	100,0	105 9	49,3	— 1,3
1950	100,0	104,8	47,8	— 1,5
1951	100,0	118,8	27,0	+ 2,3
1952	100,0	104,1	47 8	— 1,3
1953	100,0	101,5	49,5	— 0,5

The superior yielding capacity of the bearded mutant is striking. For the first time it has been shown by a mutation experiment that awns may have a positive function. A similar claim was repeatedly made earlier from comparisons between sister lines of crosses segregating in awn character or from experiments using backcrosses (*cf.* MILLER, GAUCH and GRIES, 1944; VERVELDE, 1946; SUNESON, BAYLES and FIFIELD, 1948). The positive effect of awns was mainly considered due to their ability to increase assimilation and transpiration in favour of

the size of the grains. An increase in yield, however, was not always followed by an increased seed size and this is true also in the present investigation. The only year showing a higher 1000-grain-weight of the mutant is 1951 with its heavy attack of black stem rust. The intact awns helped the grains to ripen more normally. GRANTHAM (1917) and VERVELDE (*l. c.*) suppose that the B_1-factor may also influence the tillering capacity and the length of the head.

For a country such as Sweden with advanced wheat breeding, where further progress will only be made at tremendous cost, and whose market varieties are without exception beardless, the problem of awning may be of great practical interest.

5. SHORT-STRAWED MUTATIONS.

From a practical point of view the short-strawed mutations require attention as well, since they generally have a stiffer straw. This improvement of a valuable agronomic character is caused not only by the mere shortening but generally also by an increased thickening of the straw. With the growing conditions in Svalöf, the short-strawed mutants of normal head type unfortunately experienced the same difficulty in maintaining grain yield as did the similar short-strawed but dense-eared mutants (*cf.* MAC KEY, 1954 b). This special type of growth tends to wither rapidly at maturity and thus to a decreased seed size. The yielding capacity is further generally lowered by a shortening of the length of the head. Since the ear density is unchanged, this means a reduced number of spikelets.

Apart from this negative pleiotropic effect of a straw-shortening mutation on yield, this habit is generally induced under conditions, where complete fertility and constancy are difficult to maintain. A very great part of this group of mutation, at least 80 per cent, seem to be deletions, since the homozygote is lacking or extremely rare and feeble. As to ratio-types, these mutants resemble the speltoids, which suggests that not only deficiencies but also monosomy are often involved. Such an interpretation is consistent with earlier reported causes of dwarfing (GOULDEN, 1926; HUSKINS, 1931; SEARS, 1944). The mechanism of deficiency-duplication is also represented among the cytological processes behind short-strawed mutations. The mutant 122 giving a relative grain yield of 96,6 per cent (mean of 4 trials) and a 15 cm shorter straw thus showed a frequency of multivalents in M I of the homozygote reaching 11,1 per cent (150 : 1352).

6. CHLOROPHYLL MUTATIONS.

The observation of STADLER and others which was reported in the introduction, namely, that the chlorophyll apparatus of hexaploid wheat shows a high phenotypic stability against induced mutations, is verified in the present investigation. The frequency of chlorophyll mutations at no time reached more than 1 per cent even with the highest doses of neutrons, while the comparative figures are 27 per cent in diploid barley, 26 per cent in diploid wheat, and 9 per cent in tetraploid wheat (MAC KEY, 1953).

Using GUSTAFSSON's (1940) nomenclature, the following chlorophyll mutations appeared in the present material of 6*x*-wheat: one *maculata* in Scandia III, X_2; one *maculata* and one *luteostriata* in Rival, X_2; one *tigrina* in Rival, N_2 (*cf.* Table 4). The *tigrina* type was not previously reported in wheat (*cf.* FRÖIER, 1946). It completely resembled those of barley and oats but was lethal in the seedling stage as in the diploid species. Since the same type of mutation is observed in diploid and tetraploid wheat (unpubl. results), complete analogy exists. The same holds true for the *luteostriata* type which was previously known in hexaploid wheat (WÅLSTEDT, *cit.* ÅKERMAN, 1929; ÅKERMAN, unpubl.; MAC KEY, unpubl.). The two *maculata* mutants are, however, more unique. None of them belong to the type observed in barley, *etc.* They should more accurately be called *maculescens*, since they start normally green and then spot-wise turning yellow. This decomposition of the chlorophyll occurs successively and is regulated by the age of the leaf. One and the same plant thus has leaves ranging from completely green to others whose chlorophyll only appears as a faint network around the yellow spots (Fig. 81). The decomposition does not start immediately and seems regulated by light and temperature. The *maculescens* mutant in the winter wheat is thus normally green throughout the whole autumn and winter, and it is not until the middle of April that the yellow spotting occurs, with little variation from year to year. The mutation is semi-dominant, shows genic inheritance, normal meiosis and fertility. The homozygote is characterized by a 30 per cent lower grain yield than the mother line.

7. OTHER MUTATIONS.

There are comparatively few mutations which cannot be grouped into the above mentioned classes of mutations. The main part of these other mutations, however, show phenotypical characteristics in common with some types of the earlier groups. Therefore, they may often

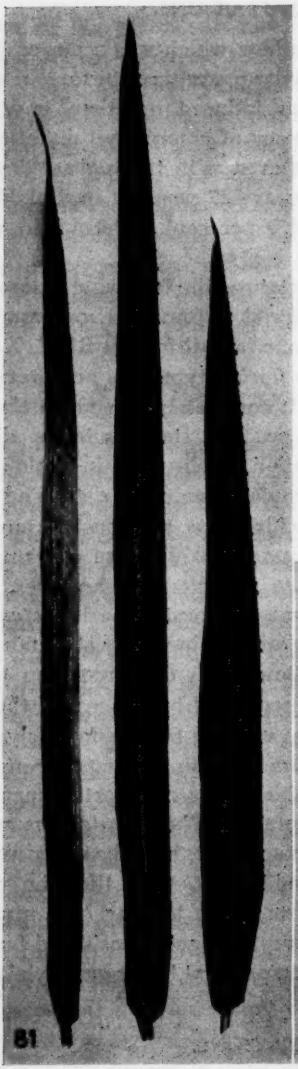


Fig. 81. Leaves of one and the same *maculescens* plant (X-mutant of Scandia III). The older the leaf the more intensive the spotty decomposition of the chlorophyll.

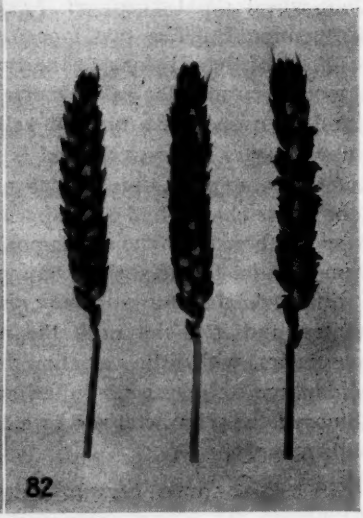


Fig. 82. From left to right: Scandia III, normal head; head of melanotic X-mutant (pseudo black chaff); head of a dense-eared X-mutant with adventitious spikelets.

be considered as »part-mutations», *i. e.*, they include changes in a smaller number of loci.

Such a type is the transition from spring to winter wheat, including 17 mutations in the Rival experiments. Considering the basic genotype, they must be induced in chromosome IX as gene mutations or interstitial deficiencies proximal to the B_1- and Q-factors. They thus show analogy with the bearded mutations with the sole exception of the events occurring closer to the centromere. Their frequency averages 0,7 per cent in X_2 and N_2, which agrees very well with 0,8 per cent for the bearded mutations. This conformity may be a further proof for a random distribution of mutations along the long arm of chromosome IX.

In addition to morphological changes, an increase or reduction of the wax cover occurs on the whole plant or parts of it. As a rule, increased waxiness is associated with internode shortening mutations but the contrary does exist. Thus among the dense-eared mutations, 1 bright green type was observed in Scandia III, X_2 and 5 in Rival, X_2 and N_2, together with 3 showing a reduced wax cover in the head region only. In the Rival material 4 bright green mutations were also found among the short-strawed group. This reduced waxiness is, however, much more common among lax-eared mutations (*cf.* p. 146). In addition, 8 bright green mutations in Rival, X_2 and N_2, were observed without any morphological deviation from type. These new mutations equal the corresponding types common in barley (*cf.* GUSTAFSSON, 1947) and, like those, are generally completely fertile and may thus possibly be simple point mutations. No detailed investigations have been made on this point, however.

Another characteristic, often observed in connection with earlier reported types of mutations, is a tendency to melanotic spotting in the head region and the upper part of the straw. This brown-coloured spottiness is often observed in connection with disturbed fertility (*cf.* p. 146) but exceptions to that rule occur. One such mutation, otherwise apparently unchanged, is presented in Fig. 82. The symptom is interesting, since a similar sign of disease is observed in several connections. According to earlier investigations (BROADFOOT and ROBERTSON, 1933; BAMBERG, 1936; HAGBORG, 1936; FRÖIER, 1938; MC FADDEN, 1939; BJÖRLING, 1946), at least eight different causes or combinations of these are found to occur. The disease may thus be produced by two different bacteria (*Xanthomonas translucens* var. *undulosa*, *Pseudomonas atrofaciens*), by four different fungi (*Alternaria tenuis*, *Helminthosporium sativum*, *Septoria nodosum*, *Puccinia graminis tritici*) or by

two types of physiological discoloration (pseudo black chaff, internodal melanism). In the present cases, microscopic studies of the necrotic tissues revealed the presence of neither bacteria nor fungi, nor could any appearance of exudation or pycnidia be observed. Nor are the detailed symptoms consistent with those of internodal melanism. They agree, however, very well with those described for pseudo black chaff which is considered as a varietal characteristic regulated by environmental conditions. Since it has not been pointed out earlier, it should be mentioned that this type of discoloration often occurs in occasional segregates from crosses between widely separated parents, *i. e.*, conditions where disturbances occur similar to those in connection with mutagenic treatments. The induced pseudo black chaff is studied in only one Scandia III X-mutant with non-association of other characteristics. This melanotic mutant (Fig. 82) had a normal meiosis and was characterized by a monomeric segregation. Since the appearance of the brown spots proved to be dependent on environmental conditions, the discoloration was observed to be recessive one year and the other year to be semi-dominant. This reaction is obvious from the following segregation tests of the F_2 after a backcross with the mother line:

Year of analysis	Normal plants	Melanotic plants	Total	χ^2	P-value for 3 : 1
1946	29	89	118	0,01	0,92
1950	317	123	440	2,05	0,15

Finally, another interesting mutation ought to be mentioned, *i. e.*, another physiological imitation of a parasitic disease. The mutant occurred in Rival, N_2 and is characterized by similar symptoms as those of the foot rot or eyespot disease (*Cercosporella herpotrichoides, etc.*). The signs of the disease appear shortly after heading with a necrotic brown discoloration of the lowest part of the culms. In a very early stage, the culms drop to the ground in a disorderly fashion and the seed becomes badly shrivelled. The intensity of the disease is aggravated if parasitic foot rot is present, indicating not only a cumulative effect but also a hypersensitiveness.

VI. DISCUSSION.

In the earlier attempts to unravel the genetic composition of hexaploid wheat, the research work was mainly confined to crosses and syntheses of species. The artificial induction was not used in order to

clarify the problem. The present investigation illustrates the degree of gene reduplication and corroborates the synthetical studies. The limited but manifest variation in types of mutations verifies that the 6*x*-wheat can hardly be strictly autopolyploid but at the same time it shows that the three component genomes cannot differ too much. The observable mutability coincides quite well with the morphological differences between the supposed parental genomes (differences in straw height, head type, brittleness of rachis, glume shape, *etc.*; *cf.* Fig. 3).

The high buffering, and the good tolerance to chromosomal rearrangements consequent to gene reduplication, give the polyploid another mode of mutating than that of the diploid. Since an elimination or profound change of fundamental factors, *e. g.*, the ability to produce chlorophyll, at the diploid level will result in non-viability, such mutants may not partake in the formation of amphidiploids. This circumstance must mean a polymerization of all such factors and thus a phenotypic stabilization at the polyploid level. The phenotypically observable mutability of a polyploid is concentrated on more peripheral characters than in a diploid. Except for the occurrence of awns and adventitious spikelets, all mutations of 6*x*-wheat can be classified as shortening, lengthening, thickening or thinning of the already existing organs, or as mere physiological changes. None of the mutations show radical organic alterations or innovations as in the diploid. Here the whole architecture of the plant may be changed. The mutation may break the morphological frame of the species. These so-called drastic mutations have repeatedly been observed in X-rayed barley (STADLER, 1931; GUSTAFSSON and ÅBERG, 1940; GUSTAFSSON, 1946, 1947, 1951; STUBBE and BANDLOW, 1947). They were even more frequent in the experiments of the present writer using the more vigorously acting neutrons. Fig. 83, representing an N-mutant of Bonus barley, may suffice to illustrate this. The mutant has branched stems, shortened leaves without ligula, auricles and differentiation in sheaths and blades, spikes with a very reduced number of flowers, hooded-like lemma, *etc.* This wider morphological mutability of the diploid must be of general application. The experiences of mutation experiments with other diploids confirm this, *e. g.*, with *Triticum monococcum* and *aegilopoides* (L. SMITH, H. KIHARA, K. YAMASHITA), with *Zea mays* (L. J. STADLER) and with *Antirrhinum majus* (H. STUBBE).

The high buffering characteristic of the hereditary mechanism of a polyploid organism, if compared with that of a related diploid, results in different evolutionary trends. As to the subsequent morphological

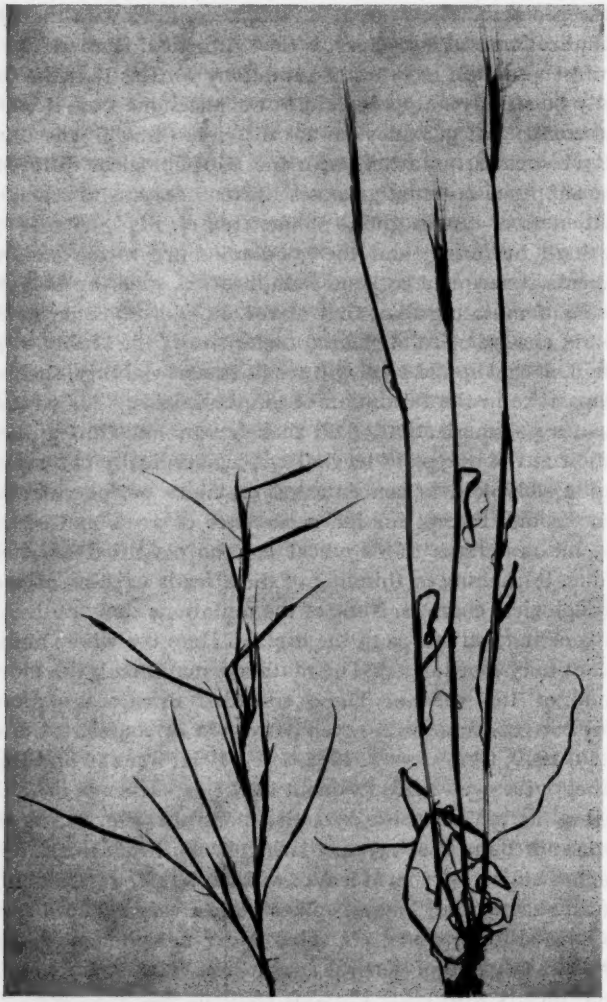


Fig. 83. An example of a drastic N-mutant of barley with the mother type (Bonus) to the right.

differentiation, polyploidy has brought the organism into a blind alley, the breadth and depth of which are determined by the degree of polyploidy and the similarity of the added genomes. Of course, the differ-

entiation here follows the same principles as in diploids, but gross chromosomal alterations, including deletions, must be of more importance at the polyploid level. This consideration is also of great interest in plant breeding. Since the vast majority of induced mutations seem to be rather obvious changes in the chromosome structure, mutation breeding should warrant even more success, theoretically, in a polyploid than in a diploid.

If the evolution with transition to polyploidy has stopped at a more stable form, the buffering mechanism, on the other hand, permits more subtle differentiations. The high tolerance to chromosomal rearrangements and the better possibilities to use them for the purpose of recombination, the higher absolute mutation capacity and the greater chances for spontaneous chromosomal alterations through the increased complications at meiosis, all contribute to a richer genotypic variation, — but restricted within narrower phenotypic limits. Polyploidy does permit a wider and more delicate ecological differentiation within certain morphological frames. Since a diploidization of the raw polyploid, *i. e.*, a tendency to chromosomal dissimilation, generally occurs, this difference in behaviour of the diploid and the polyploid will, however, be successively diminished.

The above conclusion, based on the present results of induced mutations at the diploid and polyploid levels, coincides very well with the conditions in nature. In *Triticum*, the 6x-group is morphologically more homogeneous than the 4x-group, but the first-mentioned one shows a wider ecological adaptation. If the comparison is brought down a step further to the diploids, this tendency is accentuated. The morphological differentiation is here so great that we cannot absolutely decide where a generic limit can be fixed in the Linnean system. The general idea is that the genera *Triticum*, *Aegilops*, *Agropyron*, *Secale*, and *Haynaldia*, at least, should be considered in this connection. From the above it is thus obvious that conclusions can be drawn from pure mutation experiments, corresponding with, verifying and enlarging upon those reached from quite other starting-points (*cf.* STEBBINS, 1950; HAGBERG, 1953). Here as well as there, however, the danger of too wide a generalization must always be borne in mind.

The pronounced conformity between spontaneous and induced mutating in 6x-wheat is quite expected when considering the high phenotypical buffering of the germ plasm. Thus, in wheat it is not enough to just study observable mutations and to prove their identity, if, as did STADLER (1932), one wants to check the actual conformity

between spontaneous and induced mutating. Phenotypically, identifiable mutations in 6*x*-wheat are too small and one-sided a group to be representative for such a discussion. A similar judgment must, however, also be made for the material upon which STADLER himself based his opinion that a pronounced dissimilarity exists, and which resulted in his negative attitude towards mutation experiments as a method of plant improvement (STADLER, 1930). The spontaneous mutations, their mechanism and distribution over the whole germ plasm cannot be evaluated by an analysis of a very uniform and well-established control material only. Such a basis is not representative. It does not consider the important source of spontaneous mutations that crosses are (*cf.* p. 100). If one is to generalize from the present experiences with the speltoids, — and this does not seem to be completely unwarranted, — the different sources of spontaneous mutability should *inter se* be more distinct than if a comparison is made with the induced mutations. At least when considering the whole set of mutagens available, the artificially induced mutations should be accepted as being better able to represent the average than either of the two sources of spontaneous origin. Since the mutations induced by physical mutagens seem to correspond less with the spontaneous mutations obtained in an established strain than with those produced by crossing, experiments of STADLER's type will easily lead to false conclusions. It is likely that there are more similarities than dissimilarities between spontaneous and induced mutations. It is our eagerness to obtain obvious effects which entices us to apply too heavy doses, where the minor events of mutations become obscured, phenotypically restrained or completely annihilated. We get a false idea of the ability of our mutagens to imitate nature.

From the point of view of plant breeding, this argumentation also implies a certain well-grounded criticism of the way, in which the present mutation experiments were carried out. It is not enough with the risk for a surplus of destructive mutations with doses which, according to FRÖIER, GELIN and GUSTAFSSON (1941), induce visible chromosomal disturbances in up to 75—100 per cent of the treated cells. The mere picking out of phenotypically distinct mutations hardly coincides with the aims of selection in plant breeding. The method of selection must follow the same principles as in the ordinary breeding programs. This is especially valid in the mutation breeding of polyploids, where the total frequency of induced mutations is considerably underestimated by a mere selection of phenotypically identifiable mut-

ations. In the polyploids with their high ability of buffering, these mutations can hardly be considered to represent the most valuable types for plant breeding. A considerable part of these distinct mutations are subvital, since they are induced by gross chromosomal alterations. This is evident not only from the above cytogenetical analyses but also from the fact that a very great share of the morphological mutations of 6*x*-wheat are dominant. Speltoid, dense-eared as well as the main part of lax-eared and short-strawed mutations must be considered dominant, if this term is used to classify an easily recognized departure from type when heterozygous. In the diploids, however, nearly all mutations are recessive. This pronounced difference must be due to a different ability to transmit gross chromosomal alterations through the gametophytic stage, since such a dissimilarity in reaction cannot be qualitatively gene-conditioned.

Mutation experiments of the present type do not solely give a picture of the organism's way of mutating, its relative mutability and the possibilities of improving it by induced mutations. If we consider that mutagenic treatments are merely methods of speeding up spontaneous mutating, we ought to expect not only a stimulated but also an imitated evolution. Nature both innovates and repeats. With this latter aspect, the mutation experiments should not only inform the systematist about stable and unstable characteristics of the species but should also sometimes show how to reconstruct courses of evolution which have occurred in nature. The mutation problem concerning speltoids and compactoids, extensively analysed in the present paper, thus offers both a stimulating and a puzzling analogy to the differentiation of the 6*x*-wheats belonging to *Tr. vulgare*, *Tr. compactum* and *Tr. Spelta*.

In spite of intensive investigations, where genetical, geographical, archaeological, philological and serological studies have contributed to an increased knowledge, the problem of the interrelations of the wheats mentioned is not solved. Without referring to older literature, where subjective speculations and biased information easily explain divergent ideas, one finds as many opinions as there are possibilities. MC FADDEN and SEARS (1946), for instance, persist in the oldest conception of *Tr. Spelta* as the primary hexaploid type, SCHIEMANN (1947, 1948) maintains FLAKSBERGER's (1930) stand that *Tr. vulgare* is here to be considered as the progenitor, while BERTSCH and BERTSCH (1949) state that *Tr. compactum* is the oldest hexaploid wheat.

If these differences of opinion are to be discussed and evaluated on the basis of the speltoid and compactoid mutations of *Tr. vulgare*, errors

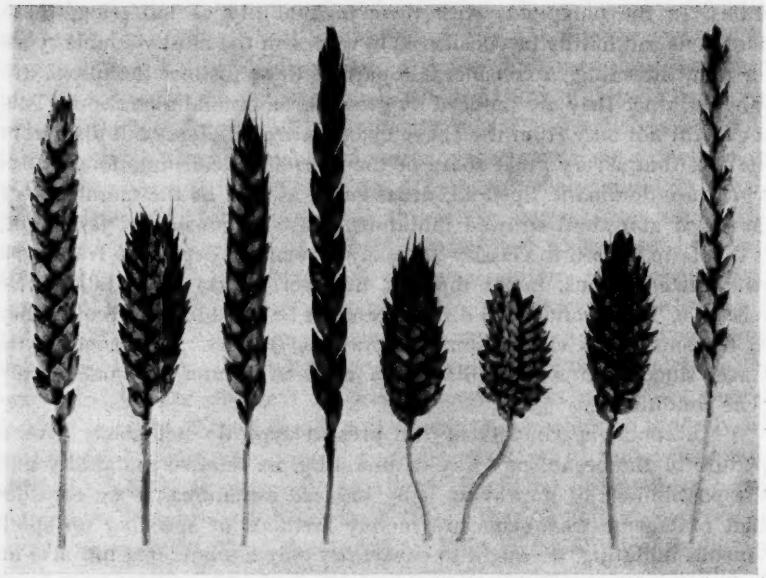


Fig. 84. The genic interrelation between compactoids, speltoids, *Tr. compactum* and *Tr. Spelta*. — From left to right: Normal head of *Tr. vulgare* var. Scandia III, compactoid 174, compactoid 174×*Tr. Spelta*, F_1 (*vulgare* type), *Tr. Spelta*, *Tr. Spelta*× *Tr. compactum*, F_1 (dense-eared spelt type), *Tr. compactum*, speltoid 174×*Tr. compactum*, F_1 (dense-eared spelt type) and speltoid 174.

must first be corrected. In the chapter on dense-eared mutations, it was definitely proved that the *compactum* and the compactoid genotype are not identical. Only the latter is able to counterbalance the speltoid mutation (Fig. 53), and it is just as important that the same holds true *vis-à-vis Tr. Spelta* (Fig. 84). The cross compactoid×*Tr. Spelta* results in a *vulgare* head type with a tough rachis. Intercrossed with *Tr. compactum*, *Tr. Spelta* gives a dense-eared spelt and the same effect is obtained, if the true spelt is substituted by the speltoid. It is evident that *Tr. compactum* and its relation to *Tr. vulgare* and *Tr. Spelta* cannot be cleared up by the compactoid mutation. Thus, BERTSCH and BERTSCH's (*l. c.*) contribution to the discussion cannot be directly evaluated here, even if their opinion of *Tr. compactum* as a synthesis of *Tr. dicoccum* and *Tr. monococcum* is impossible, since these species lack both the *compactum* factor *C* (NILSSON-EHLE, 1911) and the Q-factor.

BERTSCH and BERTSCH base their opinion on neolithic finds of

small, very round grains of wheat observed together with emmer, einkorn and barley. They consider that these grains belong to an extinct hexaploid *compactum* type, described by HEER as *Tr. vulgare antiquorum*. Since this form does not exist any longer, its hexaploidy cannot, of course, be proved, even if it seems very likely. MC FADDEN and SEARS (*l. c.*) hold it for a tetraploid in order to fit it into their hypothesis, according to which it intercrossed with *Tr. Spelta* should give rise to *Tr. vulgare* and *Tr. compactum*.

It is important to establish from Fig. 84 that compactoid 174 is not only able to counterbalance the speltoid (verified for speltoids 295, 153, 174, 298, 34, 93_1, and 35 of Table 7) but also *Tr. Spelta* (verified for var. *Duhamelianum* MAZZ. and var. *Alefeldii* KÖRN.). This ability is common to all variants of duplicated *Q*-factors and shows that the speltoid suppressing and compactoid promoting effect of chromosome IX was earlier misinterpreted. PHILIPTSCHENKO (1934), WATKINS and ELLERTON (1940), *etc.* right up to SEARS (1952 a) attribute the speltoid suppressing ability of chromosome IX to a gene *k* (or *S*) situated about 65 crossing-over units from a squarehead gene *q*, the two genes being located one on each side of the beard inhibiting factor B_1 on the same arm. The present mutation 174 already disproves this conception, since the break of chromosome IX here occurred distally to the B_1-factor. This must mean that compactoid 174 has the *Q* duplicated but only one set of *k*. At the same time, speltoid 174 must retain its gene *k*, which does not, however, fit with its phenotype. The only explanation should thus be an additional mutation, this time on the other side of B_1. Nevertheless, if this is true, compactoid 174 should not be able to counterbalance speltoid 174, nor any other beardless speltoid, which happens consistently. WATKINS' *K*-factor (1927, 1928, 1940) must be more adjacent to *Q*. In reality, they are not only close to each other, they are even identical, a fact which has already been accepted in the presentation of the data in this paper (*cf.* p. 106).

PHILIPTSCHENKO's (*l. c.*) analysis, which is responsible for the earlier misconception, also lacks a clear argumentation for the critical point. Studying a cross between *Tr. vulgare* and a *compactum* type, he observed that the head density was regulated by two pairs of genes. One was the *compactum* gene *C*, and the other a gene for squareheadedness. The latter was absent in the *compactum* parent (*cf.* Fig. 84, head Nos. 5 and 7) but was introduced by the *vulgare* parent. He considered the gene to be recessive, called it *q*, and thought that it was phenotypically active only in double dose and together with *C*, — for which reason he

wrote $Q=C+qq$. He found q to be linked to the beard inhibiting gene B_1 (=his N) and determined the distance between them to approximately 33 crossing-over units. He brought this discovery together with earlier analyses on the distance between B_1 and the locus controlling the spelt habit. This distance, determined by the cross *Tr. Spelta*×*Tr. vulgare*, was estimated by KAJANUS (1923) to be 33, by NILSSON-LEISSNER (1925) to be 35, and by WATKINS (1928) to be 28 crossing-over units, figures in good conformity to that of NILSSON-EHLE (1927) of 26, based on a beardless def-speltoid. Since the squarehead and speltoid segregates could easily be separated in speltoid crosses, PHILIPTSCHENKO presumed that the genes q and k (=his S) were situated far apart. In his diagram of the linkage group, he thus placed them on each side of B_1 instead of using the data gathered as an excellent proof of identity. As a logical consequence of this new interpretation, k and q are here incorporated into Q. The gene must be considered dominant, since it results in a distinct departure from type when heterozygous. Speltoid 295 (*cf.* p. 120) and similar cases should have q, if they really are true gene mutations.

The fact that the speltoid and the compactoid mutations are regulated by one and the same gene, strengthens the significance of WINGE's (1924) balance theory. On the other hand, it breaks down WATKINS' (*l. c.*) system of a polymeric series of K-allelomorphs which was thought to control the glume and rachis appearance of the wheat species. Q cannot, however, as WINGE suggested, be counterbalanced by one factor in the homoeologous chromosome only. A great many genes, spread over the whole germ plasm, must be considered to interfere, each of which is having a special function. They may regulate the internodal length, the shape of the glumes, the brittleness of the rachis, *etc.* Their common characteristic is that they do not influence the phenotype in the same way with Q present, as when Q is absent. This manifold counterbalancing of Q explains why speltoids are always induced by an inactivation and compactoids by a duplication of Q. The counterbalancing set of genes are too scattered to be affected by a single mutation.

From the point of view of presence or absence of the Q-factor, there is thus no difference between the speltoid and *Tr. Spelta*. They both lack Q. The difference lies in the composition of the remaining gene set involved in the interference. If genes for a brittle rachis are lacking but not those for laxness and spelting, brittleness will not occur when Q disappears. This is plainly shown by the fact that Scandia III gives tough speltoids, while Rival consistently gives speltoids as brittle

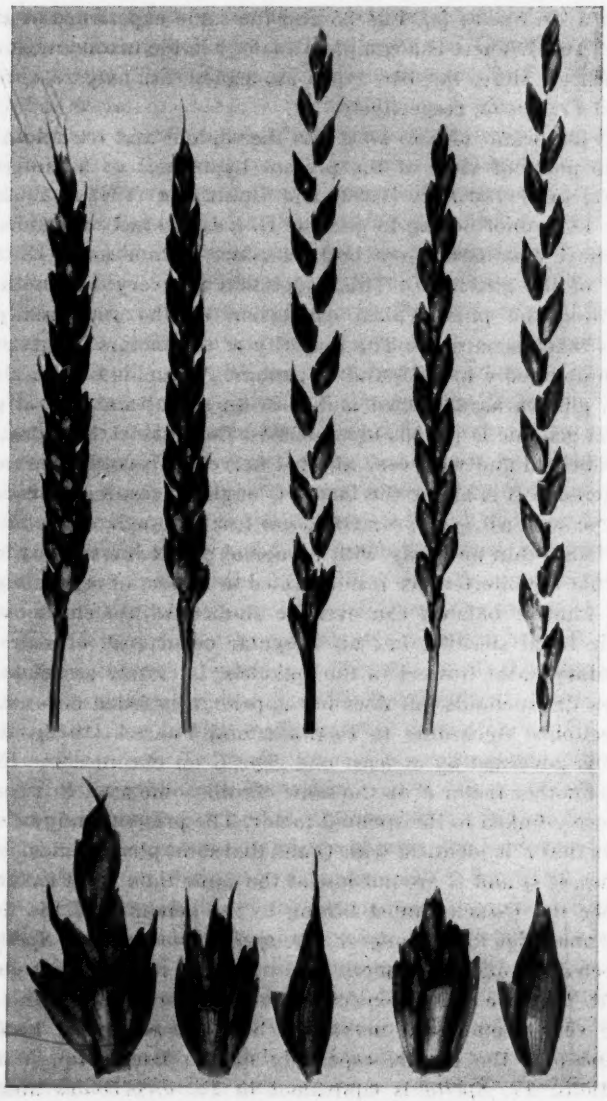


Fig. 85. Induced and natural spelt wheats. Heads of *Tr. vulgare* var. Rival, a Rival-speltoid (head unbroken and broken) and *Tr. Spelta* (ditto). Below, three spikelets (one branched analogous with *Spelta* observations of NILSSON-LEISSNER, 1925) of the speltoid and two of *Tr. Spelta.*

as many a *Tr. Spelta* (*cf.* Fig. 85 and the same experience of PHILIPTSCHENKO, 1929). There is a complete analogy in the taxonomical division of the wheat. Here, the two types are called *Tr. vulgare speltiforme* VAV. and *Tr. Spelta*, respectively.

It is important also to look into the diploid and tetraploid wheats from the point of view of the present hypothesis of an interference. According to MATSUMURA (1939) and UCHIKAWA (1942 a, 1950), chromosome IX cannot belong to genome D, *i. e.*, the last one added of the three wheat genomes. They both consider chromosome IX to be a member of the genome B. This suggestion fits very well with Fig. 3, representing the phenotypical expression of the supposed parental genomes taken separately. The majority of the factors interfering with *Q* are undoubtedly to be found in genome A (rachis breaks above the spikelet, glumes hard, keeled and with an apical and lateral pointedness) and genome D (rachis breaks below the spikelet, head lax, glumes hard, truncated and with very marked nerves). The consequence is that where genome B is alone, the factor *Q* ought to result in a compactoid phenotype such as *Agropyron triticeum* (rachis tough, dense-noded and glumes loose, thin and only with an apical pointedness). It is to be observed that the interference is not limited to factors of separate genomes only. A kind of balance can even be studied within chromosome IX. Diplontic basal sterility, *i.e.*, an irregular occurrence of more or less rudimentary basal flowers in the spikelets, is closely associated to the beardless def-speltoids but does not appear in bearded def- and monosome-speltoids. According to FRANKEL and FRASER (1948), the basal sterility is governed by at least one gene *G* on chromosome IX, hypostatic to another factor *F* on the same chromosome arm. *F* is considered to be closely linked to the speltoid factor. The present study of speltoids indicates that *F* is identical with *Q* and that some physiological irregularity occurs, if *Q* and *G* are not lost at the same time (*cf.* FRASER, 1950).

Since the *Q*-factor must belong to the genomes of the tetraploid wheats, analogies to *Tr. vulgare*, *Tr. speltiforme* and *Tr. Spelta* are to be expected among the emmers. The only difference is that genome D is absent. Thus we cannot expect the rachis to break below the spikelet, nor the very pronounced nervation, but still a marked keeling and pointedness of the glume, especially of the lateral tip. With these reservations, *Tr. Spelta* is equivalent to *Tr. dicoccoides* and *Tr. dicoccum* which also segregate *Spelta* types when crossed with *Tr. vulgare*, *i. e.*, after addition of genome D (*cf.* MALINOWSKI, 1914, 1916; KAJANUS, 1923; RAUM, 1931). *Tr. turgidum* is further to be considered

as analogous to *Tr. speltiforme*. This is evidenced by the high percentage of the tough so-called *speltoides* in the offspring of *Tr. vulgare*×*Tr. turgidum* (*cf.* p. 100). *Tr. vulgare*, finally, corresponds to *Tr. persicum* of the tetraploid series.

If the above interpretation of the phenotypical significance of the *Q*-factor is correct, the syntheses *Tr. dicoccoides*×*Aegilops squarrosa*, *Tr. durum*×*A. squarrosa* and *Tr. turgidum*×*A. squarrosa*, all resulting in a *Spelta* type (KIHARA, 1944; MC FADDEN and SEARS, 1944; KIHARA *et al.*, 1950) are just as inconclusive for the phylogenetic discussion as the above-mentioned *Spelta*-like derivatives of *Tr. vulgare*×*Tr. dicoccum* or the present *Spelta*-speltoid induced in *Tr. vulgare* var. Rival (*cf.* SCHIEMANN, 1932, 1948). An addition of genome D to a tetraploid wheat without the *Q*-factor must give the same result as when one starts with the complete interfering complex and removes the *Q*-factor. Thus the phylogenesis cannot be proved by genetical data only. There are, however, a restricted number of explanations that have to be checked against known facts of a non-genetic nature. One of the above-cited, experimentally verified courses must certainly have occurred in the evolutionary differentiation of *Tr. vulgare* and *Tr. Spelta*. We must, however, in this consideration also include the possibility that *Tr. Spelta* may have originated from *Tr. compactum*×*Tr. dicoccum*. On the other hand, it seems justifiable to refuse a hypothesis which might be built upon information from SMITH, HUSKINS and SANDER (1950) that the long IX-arm includes an inverted repeat. This implies that the *Q*-factor could be a duplication similar to *Bar* in *Drosophila*. If that is so, *Tr. vulgare* could have arisen from *Tr. Spelta* through a simple duplication, which is quite in conformity with SCHRIMPF's idea (1951) of an original identity between genomes A and B. That would imply that the bearded speltoids, at least, would not be identical with *Tr. Spelta* as to the *Q*-dosage. The apparent absence of a position effect for *Q* (*cf.*, however, LEWIS, 1951) and the ability of the compactoid to counterbalance both types to the same degree thus speak against this proposal. In addition, the idea is contradicted by historical and geographical data.

Of the remaining suggestions, that of MC FADDEN and SEARS (*l. c.*) seems least plausible from a non-genetic standpoint. FLAKSBERGER (1930) and SCHIEMANN (1932, 1947) computed overwhelming geographical, archaeological and philological proofs of *Tr. Spelta* as a form endemic of the North-Alpine region and of late- or post-neolithic origin. It is true that *Tr. dicoccum* was grown in this region at that time, but the other necessary component according to EIG (1919) has quite an-

other distribution area, *i. e.*, the region west, south and especially east of the Caspian Sea. Thus, *Aegilops squarrosa* grows in the region which VAVILOV (1923) designates as the gene centre of the 6*x*-wheat and where *Tr. persicum* VAV. (4*x*-wheat with *Q*-factor) also is endemic (ZHUKOVSKY, 1923). It seems, then, more logical to consider *Tr. persicum* or some analogous tetraploid as a component already in the hexaploidization instead of introducing this type, as do MC FADDEN and SEARS (*l. c.*) in the subsequent speciation at the hexaploid level. In order to fit their explanation with archaeological data, these scientists in addition must put down *Tr. antiquorum* as more or less similar to *Tr. persicum*, the former being considered a hexaploid by other authorities (SCHIEMANN, 1932; BERTSCH and BERTSCH, 1949). If the *Q*-factor originates from *Agropyron triticeum* or some related diploid, one has to consider the original tetraploid to be a *Q*-bearing wheat, and then spelting processes to have occurred independently at the tetraploid as well as at the hexaploid level. If one believes that genome B is originally identical with genome A, the *persicum* type must of course be considered as a secondary product. Such a type may occur from *Tr. dicoccoides* (or another non-*Q* 4*x*-wheat) × *Agropyron triticeum* but neither is there anything to prevent the appearance of more than one tetraploidization. However that may be, a *Q*-bearing type must be involved in the synthesis of the hexaploid wheat. Otherwise it will be difficult to explain the non-existence of *Tr. Spelta* in the mentioned gene centre.

The original hexaploid wheat undoubtedly had a more or less fragile rachis. This assumption is favoured not only by the fact that two genomes (A and D) out of three contribute genes for brittleness but also by KIHARA and LILIENFELD's (1948) cross *Tr. persicum* × *Aegilops squarrosa* resulting in a brittle triploid. RAUM's (1931) brittle so-called *vulgoides*, rare segregates in species crosses including brittleness, offer further evidence. The genes for a fragile rachis disappeared successively and at a certain stage they became completely counterbalanced by the *Q*-factor. Since the *compactum* factor *C* has a similar ability (valid only for the brittleness and not for the spelting), the first step towards a hexaploid wheat with a tough rachis may have been *Tr. compactum*. This type seems also to have preceded *Tr. vulgare* in ancient centres of culture (*cf.* SCHIEMANN, 1932; BERTSCH and BERTSCH, 1949). Only later did the elimination of genes for brittleness reach that point where *Q* alone was able to counterbalance the rest. Such a process must imply a considerable improvement, since the factor *C* keeps down the seed size. The elimination of the remaining genes for brittleness continues to this

very day, which is amply shown by the speltoids in Rival and Scandia III.

The spelting processes, registered by the taxonomist in his list of hexaploid wheat taxa, have probably occurred in different stages of the evolution outlined above. The pronounced brittleness of *Tr. Spelta* in contrast to *Tr. speltiforme* favours such an opinion. *Tr. Spelta* undoubtedly descends from *Tr. compactum*, or to be more explicit, from its variant *Tr. antiquorum*, where the gene regulation of a tough rachis must have been definitely more primitive than in *Tr. vulgare* from which *Tr. speltiforme* certainly comes. As evident from the above, the two spelting processes, one in Central Europe, one in South-Western Asia, may have arisen either by mutation or by recombination. Which of the two processes occurred, cannot be definitely decided. Considering *Tr. speltiforme*, the first alternative seems much more likely, since the *Q*-factor is known to delete easily. Considering *Tr. Spelta*, however, one has to presuppose two mutations, one in chromosome IX affecting *Q* and one in chromosme XX affecting *C*. Thus, it seems more likely that *Tr. Spelta* originated from the cross *Tr. compactum* (*antiquorum*) $\times$ *Tr. dicoccum*. In addition, this opinion is favoured by archaeological evidences of the two parental species already being grown together in the North-Alpine region during the neolithic age (SCHIEMANN, 1932; BERTSCH and BERTSCH, 1949). Such a combination may very well explain the different collar type at glume base of *Tr. Spelta* and the speltoid (*cf.* WATKINS, 1928), as well as it may result in an increase of genes for brittleness. A cross of these two species may also tend to increase the similarity of homoeologous chromosomes and thus their chance to interpair. The high frequency of anaphase bridges in *Tr. Spelta* observed by SANCHEZ-MONGE (1949), with an interpretation similar to the one in the present paper (*cf.* Table 11), fits with such an assumption. This evidence, however, is not conclusive, since it may just as well be used to prove that *Tr. Spelta* is a product of a deficiency-duplication.

Strong support for the present idea of *Tr. Spelta*'s origin from *Tr. compactum* is obtained from THOMPSON and ROBERTSON's (1930) meiotic analysis of crosses between the hexaploid wheats here under discussion. They studied the frequency of univalents in M I, A I and A II and found it to be 4,0—4,4 per cent in pure *Tr. vulgare*, *Tr. compactum* and *Tr. Spelta*. In the F_1, these rates increased to 42,0 per cent when crossing *vulgare* $\times$ *Spelta*, to 34,0 per cent for *vulgare* $\times$ *compactum* but only to 12,5 per cent for *Spelta* $\times$ *compactum*. The higher affinity of the

last cross, though misinterpreted by the original investigators, must indicate a closer interrelation.

If the above outline of the more important differentiation of 6*x*-wheat is correct, the recent boundaries drawn by taxonomists within this group of the genus *Triticum* appear less fortunate. In one case, a loss of the factor *Q* will not exclude the mutant from remaining a *vulgare* type. The speltoid induced in Scandia III is to be classified as a *Tr. vulgare speltiforme*. In another case, a new species is obtained. Taxonomically, the speltoid out of Rival is a *Tr. Spelta*. Further, the decisive characteristic separating *Tr. vulgare* and *Tr. compactum* is governed by one factor (*C*) only. The same obviously holds true for *Tr. vulgare* and *Tr. Spelta*, where they key gene is *Q*. In the former case an incorporation has been suggested but not in the latter (*cf.* SCHIEMANN, 1948). That such a classification must be even less adequate than the old one is obvious when considering chromosomal affinity and phylogenetic interrelations.

Acknowledgments. — My sincere thanks are due to my teachers in Genetics, Professor G. TURESSON, Director of the Institute of Plant Systematics and Genetics, Royal Agricultural College of Sweden, and Professor A. MÜNTZING, Director of the Institute of Genetics, University of Lund. I am also greatly indebted to my principal, Professor Å. ÅKERMAN, Director of the Swedish Seed Association, for his interest, stimulating discussions and for all the working facilities granted me. I wish to offer my special thanks to Professor Å. GUSTAFSSON, Head of the Genetics Department, Swedish Forest Research Institute, for his unfailing interest, valuable comments and stimulating encouragement. Drs. O. TEDIN, A. HAGBERG and N. NYBOM, as well as other colleagues of the Svalöf and Lund institutions, are gratefully thanked for many interesting and profitable discussions. Very sincere thanks also to Dr. E. R. SEARS, University of Missouri, Columbia, Mo., U. S. A., for a valuable criticism and information on an important point of my work.

My most hearty thanks are due to Mr. LLOYD H. PERSON, Saskatoon, Sask., Canada, for his valuable linguistic correction of my manuscript.

Finally, I also gratefully acknowledge the financial support from the A. W. BERGSTEN Foundation through the Royal Swedish Academy of Agriculture and its Secretary, Professor R. TORSSELL, who followed my work with unceasing interest.

Svalöf, December, 1953.

SUMMARY.

The paper presents a study of the observable mutability in 6*x*-wheat and, for comparison, 2*x*-barley, where dormant seeds were treated with successively increasing doses of X-rays and fast neutrons. Owing to their high non-genetic lethality, X-rays were not able to expose differences in the mutability of a diploid and a polyploid organism (Fig. 17). The more purely genetically operating neutrons, however, revealed that wheat in spite of its polyploidy and consequent pronounced gene reduplication, gave a decidedly higher frequency of observable mutations. While the maximum number of mutations in 2*x*-barley reached 50 per cent calculated per surviving N_1 plant, values over 160 per cent were obtained in 6*x*-wheat without any indication of the maximum being reached. The higher observable mutation frequency of the 6*x*-wheat is undoubtedly due more to the better tolerance to chromosome disturbances and the high recombining ability as a consequence thereof, than to the mere increase in the number of genes. The induction of mutations in the polyploid shows a pronounced grouping effect in contrast to the diploid.

The »diploid sector» of the germ plasm of common wheat is clearly restricted and coincides very well with the differences of the supposed parental genomes. In fundamental factors, *e. g.*, the ability to produce chlorophyll, there is a high buffering ability. The phenotypically observable mutability is concentrated on more peripheral characters than in a diploid. A greater event also seems necessary for a phenotypic penetration of the mutation. While the induced vital mutations in a diploid are nearly all recessive, the main part of those in common wheat proved to be dominant or semi-dominant. As a consequence, mutations can already be registered in wheat in the first generation (Fig. 1). The different reaction to mutating gives the polyploid a different evolutionary trend than that of a diploid. Polyploidy restricts the organism to a narrower morphological frame but permits a richer and more subtle differentiation within this frame.

The most common phenotypically identifiable mutations in common wheat are speltoid, dense-eared, lax-eared and short-strawed mutations. Here, as well as in other mutations, the impulses were proved to include point mutations, visible deficiencies, deficiency-duplications, duplications, aneuploidy, *etc.* Dense-eared, lax-eared and short-strawed mutations were each proved to be regulated by several different loci, while speltoid, compactoid and bearded mutations as well as transi-

tions from spring to winter habit all proved to be monofactorially regulated in the present material.

Chromosome IX was used as test chromosome in a study of induced and spontaneous mutating. There was no indication of a non-random distribution of breaks or exchanges along its long arm, and the effect on vitality proved proportional to the size of the deleted segment when a speltoid was produced (Figs. 76 and 77). The mutability of chromosome IX, as indicated by the speltoid mutation, increased considerably by mutagenic treatments and also by mere intercrossing of established varieties. The type of mutation mechanism proved to be most different when the two sources of spontaneous mutating were compared (Table 10). The induced mutations held an intermediate position with no significant difference between X-rays and fast neutrons. The consequence of these results with regard to the discussion of identity between induced and spontaneous mutations is obvious.

The study of chromosome IX permitted a revision of the speltoid and compactoid problem. Speltoids can be produced by simple deficiency, deficiency-duplication and probably also by gene mutation and chromosome substitution. The old classification into type A, B and C has been substituted by a regrouping into two groups only, deficiency-speltoids (def-type) and monosomic speltoids (monosome-type), since a boundary between type A and C was proved to be untenable. The spontaneous def-speltoids are mainly a consequence of the inherent gene reduplication of a polyploid; they cannot be explained by assuming an unstable point in the germ plasm. Compactoids can be produced by chromosome substitution, chromosome addition, simple duplication, deficiency-duplication or isochromosome formation. Table 13 gives the proposed denomination of the different types.

Speltoids can only occur in *Tr. vulgare* by an inactivation or loss of a certain factor, compactoids only by a duplication of the very same factor, called *Q*. Other possibilities do not exist. The proof of an identity between PHILIPTSCHENKO's squarehead gene *q* and WATKINS' spelting gene *k* breaks down the latter's genetic system of wheat classification. Instead, a hypothesis suggesting an interference between *Q* and a whole set of other genes is introduced and the speciation of wheat is discussed on this basis. According to this hypothesis, the synthetic *Tr. Spelta* from *Tr. dicoccoides*×*Aegilops squarrosa* crumbles as a proof in the attempt to establish the spelt as the primary 6*x*-wheat. *Tr. Spelta* as a product of recombination from *Tr. vulgare*×*Tr. dicoccum* or as a mere mutation in *Tr. vulgare* is just as inconclusive. *Tr. Spelta* is here supposed to

originate from the cross *Tr. compactum* (*antiquorum*) × *T. dicoccum*. On the other hand, *Tr. vulgare speltiforme* should generally occur by mere mutating.

LITERATURE CITED.

1. AASE, H. C. 1946. Cytology of cereals. II. — Bot. Rev. 12: 255—334.
2. ALLSOPP, C. B. 1948. Theories of the biological actions of ionizing radiations. — Brit. Jour. Radiol. 21: 72—74.
3. ANDERSON, E. G., LONGLEY, A. E., LI, C. H. and RETHERFORD, K. L. 1949. Hereditary effects produced in maize by radiations from the Bikini atomic bomb. I. Studies on seedlings and pollen of the exposed generation. — Genetics 34: 636—649.
4. ARNASON, T. J., PERSON, C. O. and NAYLOR, J. M. 1952. Radiation-induced mutations in wheat and barley. — Can. Jour. Bot. 30: 743—754.
5. AUERBACH, C. 1949. Chemical inductions of mutations. — Proc. 8th Int. Congr. Genet. (Hereditas, Suppl. Vol.): 128—147.
6. BAMBERG, R. H. 1936. Black chaff disease of wheat. — Jour. Agr. Res. 52: 397—417.
7. BERTSCH, K. und BERTSCH, F. 1949. Geschichte unserer Kulturpflanzen. — Wiss. Verlagsges. M. B. H., Stuttgart, 268 pp. (1. Auflage, 1947.)
8. BJÖRLING, K. 1946. Melanism hos olika vetesorter. — Växtskyddsnotiser 1946, No. 6: 86—91.
9. BONNIER, G. och TEDIN, O. 1940. Biologisk variationsanalys. — Sv. Bokförl., Stockholm, 325 pp.
10. BOSHNAKIAN, S. 1922. The genetics of squareheadedness and of density in wheat, and the relation of these to other characters. — Ithaca Univ. Memoir 53: 801—882.
11. BROADFOOT, W. C. and ROBERTSON, H. T. 1933. Pseudo-black chaff of Reward wheat. — Sci. Agr. 13: 512—514.
12. BYNOV, F. A. 1938 a. Les mutations de *Triticum vulgare* provoquées par un courant électrique. — Trav. Jard. Bot. Univ. Moscow 2: 17—35.
13. — 1938 b. La centrifugation comme facteur de mutations. — Trav. Jard. Bot. Univ. Moscow 2: 36—46.
14. CALDECOTT, R. S., FROLIK, E. F. and MORRIS, R. 1952. A comparison of the effects of X-rays and thermal neutrons on dormant seeds of barley. — Proc. Nat. Acad. Sci. USA. 38: 804—809.
15. CALDECOTT, R. S. and SMITH, L. 1952. A study of X-ray-induced chromosomal aberrations in barley. — Cytologia 17: 224—242.
16. CAMARA, A. 1936. Notas sobre espeltoides. — Rev. Agron. Lisboa 24: 301—318.
17. — 1945. Variabilidade cariotípica dos trigos hexaploides. — Las Ciencias 10: 1—19.
18. CAMARA, A. e RODRIGUES, C. 1946. Contribuição para o estudo citológico dos espeltoides do tipo γ. — Agr. Lusitana 8: 269—290.
19. CATCHESIDE, D. G. 1948. Genetic effects of radiations. — Adv. Genet. 2: 271—358.
20. COFFMAN, F. A. 1924. Supernumerary spikelets in Mindum wheat. — Jour. Hered. 15: 186—192.

21. DARLINGTON, C. D. 1936. The limitation of crossing-over in *Oenothera*. — Jour. Genet. 32: 343—352.
22. — 1937. Chromosome behaviour and structural hybridity in the *Tradescantiae*. II. — Jour. Genet. 35: 259—280.
23. — 1940. The origin of iso-chromosomes. — Jour. Genet. 39: 351—361.
24. DARLINGTON, C. D. and UPCOTT, M. B. 1941. Spontaneous chromosome change. — Jour. Genet. 41: 297—338.
25. DELAUNAY, L. 1930. Die Chromosomen-Aberranten in der Nachkommenschaft von röntgenisierten Ähren einer reinen Linie von *Triticum vulgare albidum* ALL. — Zschr. ind. Abst. Vererb. 55: 352—355.
26. — 1931. Resultate eines dreijährigen Röntgenversuches mit Weizen. — Der Züchter 3: 129—137.
27. — 1932. The X-mutations in wheat. — Bull. Lab. Genet. Leningrad 9: 173—180.
28. — 1934. Experimentell erzeugte Mutationen bei Weizen. — Verlag Ukr. Akad. landw. Wiss., 56 pp. (ref. Res. Genet. 10: 164—165).
29. DEMPSTER, E. R. 1941. Dominant *vs.* recessive lethal mutation. — Proc. Nat. Acad. Sci. USA. 27: 249—250.
30. DERMEN, H. 1947. Periclinal cytochimeras and histogenesis in cranberry. — Amer. Jour. Bot. 34: 32—43.
31. DIX, W. 1934. Züchtungsversuche mit Getreide. — Landw. Jahrb. 79: 335—369.
32. DUCELLIER, L. O. 1923. L'hybridation du blé en Algérie. Formes speltoïdes et durelloïdes. — Bull. Soc. Hist. Nat. du Nord 14: 164—172.
33. DUMON, A. C. 1931. Speltoïden en compactoïden bij rassen van *Triticum vulgare*. — Natuurwetenschappelijk T. 13: 101—104.
34. EHRENBERG, L., GUSTAFSSON, Å., LUNDQUIST, U. and NYBOM, N. 1953. Irradiation effects, seed soaking and oxygen pressure in barley. — Hereditas XXXIX: 493—503.
35. EHRENBERG, L. and NYBOM, N. 1952. Chemical and biological effects of neutrons and X-rays. — Hereditas XXXVIII: 481—501.
36. EIG, A. 1929. Monographisch-kritische Übersicht der Gattung *Aegilops*. — Rep. spec. nov. regni veg., Beihefte 50: 1—228.
37. ELLERTON, S. 1939. The origin and geographical distribution of *Triticum sphaerococcum* PERC. and its cytogenetical behaviour in crosses with *T. vulgare* VILL. — Jour. Genet. 38: 307—324.
38. FANO, U. 1943. Neutron-induced lethals in *Drosophila*. — Genetics 28: 74—75.
39. — 1944. Experiments on mutations induced by neutrons in *Drosophila melanogaster* sperms. — Genetics 29: 361—369.
40. FLAKSBERGER, C. 1930. Ursprungszentrum und geographische Verbreitung des Spelzes (*Triticum spelta* L.). — Angew. Bot. 12: 86—99.
41. FONDARD et CABASSON. 1939. Influence des rayons X et des ondes courtes. — Compt. Rend. Hebd. Séances Acad. Agric. France 25, No. 13: 506—509.
42. FRANKEL, O. H. 1949. A self-propagating structural change in *Triticum*. — Heredity 3: 163—194, 293—317.
43. FRANKEL, O. H. and FRASER, A. S. 1948. Basal sterile mutants in speltoid wheat. — Heredity 2: 391—397.
44. FRASER, A. S. 1950. Basal sterility in wheat. — Nature 165: 653—654.
45. FREISLEBEN, R. und LEIN, A. 1943. Vorarbeiten zur züchterischen Auswertung röntgeninduzierter Mutationen. II. Mutationen des Chlorophyllapparates als

Testmutationen für die mutationsauslösende Wirkung der Bestrahlung bei Gerste. — Zschr. Pfl.-zücht. 25: 255—283.

46. FRÖIER, K. 1938. Brunfläcksjuka (*Bacterium translucens* var. *undulosum*) och dess angrepp på olika vetesorter. — Beretn. N. J. F. Kongr. Uppsala: 536—543.
47. — 1946. Genetical studies on the chlorophyll apparatus in oats and wheat. — Hereditas XXXII: 297—406.
48. FRÖIER, K., GELIN, O. and GUSTAFSSON, Å. 1941. The cytological response of polyploidy to X-ray dosage. — Bot. Notiser 1941: 199—216.
49. FRÖIER, K. and GUSTAFSSON, Å. 1944. The influence of seed size and hulls on X-ray susceptibility in cereals. — Hereditas XXX: 583—589.
50. GELIN, O. 1941. The cytological effect of different seed-treatments in X-rayed barley. — Hereditas XXVII: 209—219.
51. GILES, N. H. 1940. The effect of fast neutrons on the chromosomes of *Tradescantia*. — Proc. Nat. Acad. Sci. USA. 26: 567—575.
52. — 1943. Comparative studies of the cytogenetical effects of neutrons and X-rays. — Genetics 28: 398—418.
53. GOODSPEED, T. H. 1929. The effects of X-rays and radium on species of the genus *Nicotiana*. — Jour. Hered. 20: 243—259.
54. — 1937. Significance of cytogenetic alterations induced by high frequency radiation in *Nicotiana* species. — Cytologia, FUJII Jub. Vol.: 961—966.
55. GOODSPEED, T. H. and AVERY, P. 1930. Nature and significance of structural chromosome alterations induced by X-rays and radium. — Cytologia 1: 308—327.
56. GOODSPEED, T. H. and OLSON, A. R. 1928. The production of variation in *Nicotiana* species by X-ray treatment of sex cells. — Proc. Nat. Acad. Sci. USA. 14: 66—69.
57. GOULDEN, C. H. 1926. A genetic and cytological study of dwarfing in wheat and oats. — Minn. Agr. Exp. Sta., Tech. Bull. 33, 37 pp.
58. GRANHALL, I. 1943. Genetical and physiological studies in interspecific wheat crosses. — Hereditas XXIX: 269—380.
59. GRANTHAM, A. E. 1917. The tillering of winter wheat. — Del. Agr. Exp. Sta. Bull. 117.
60. GRAY, L. H. 1950. Electrons, neutrons, and alpha particles. — Biophysical Research Methods (editor: F. R. UBER), New York, Chapter 15: 491—560.
61. GUSTAFSSON, Å. 1940. The mutation system of the chlorophyll apparatus. — Lunds Univ. Årsskr. N. F. Avd. 2, Bd 36, Nr. 11, 40 pp.
62. — 1941. Mutation experiments in barley. — Hereditas XXVII: 225—242.
63. — 1946. Drastic morphological mutations in barley. — Hereditas XXXII: 120—122.
64. — 1947. Mutations in agricultural plants. — Hereditas XXXIII: 1—100.
65. — 1951. Mutationer i växtförädlingens tjänst. — Svensk Växtförädling (editors: Å. ÅKERMAN *et al.*), Natur & Kultur, Stockholm, Bd 1: 659—676.
66. GUSTAFSSON, Å. and ÅBERG, E. 1940. Two extreme X-ray mutations of morphological interest. — Hereditas XXVI: 257—261.
67. HAGBERG, A. 1953. Further studies on and discussion of the heterosis phenomenon. — Hereditas XXXIX: 349—380.
68. — 1954. Cytogenetic analysis of *erectoides* mutations in barley. — Acta Agric. Scand. 4 (in the press).

69. HAGBORG, W. A. F. 1936. Black chaff, a composite disease. — Can. Jour. Res. C 14: 347—359.
70. HEDGRAN, A. 1948. Koincidensmetoden. — Kärnfysik (Vetenskapsakad. Forskn.-inst. Fysik), Stockholm, Chapter 7, 17 pp.
71. HILLARY, B. B. 1940. Uses of the Feulgen reaction in cytology. II. New techniques and special applications. — Bot. Gaz. 102: 225—234.
72. HOLLINGSHEAD, L. 1932. The occurrence of unpaired chromosomes in hybrids between varieties of *Triticum vulgare*. — Cytologia 3: 119—141.
73. HUSKINS, C. L. 1927. Genetical and cytological studies of fatuoid oats and speltoid wheats. — Verh. 5. Int. Kongr. Vererb.-wiss. (Suppl. Zschr. ind. Abst. Vererb.) 2: 907—916.
74. — 1928. On the cytology of speltoid wheats in relation to their origin and genetic behaviour. — Jour. Genet. 20: 103—122.
75. — 1931. A cytological study of VILMORIN's unfixable dwarf wheat. — Jour. Genet. 25: 113—124.
76. — 1932. Factors affecting chromosome structure and pairing. — Trans. Roy. Soc. Can. Sect. V 26: 17—28.
77. — 1933. The origin and significance of fatuoids, speltoids, and other aberrant forms of oats and wheat. — Proc. World's Grain Exh. Conf. 2: 1—6.
78. — 1941. Polyploidy and mutations. — Amer. Nat. 75: 329—344.
79. — 1946. Fatuoid, speltoid and related mutations of oats and wheat. — Bot. Rev. 12: 457—514.
80. HUSKINS, C. L. and HEARNE, E. M. 1933. Meiosis in asynaptic dwarf oats and wheat. — Jour. Roy. Microscop. Soc. 53: 109—117.
81. HUSKINS, C. L. and SANDER, G. F. 1949. Mutations in polyploid cereals I. Introductory outline. — Can. Jour. Res. C 27: 332—347.
82. HÅKANSSON, A. 1930. Zytologische Beobachtungen an s. g. Speltoidheterozygoten beim Weizen. — Svensk Bot. Tidskr. 24: 44—57.
83. — 1931. Die Chromosomenzahl von Speltoidheterozygoten, die aus s. g. Subcompactum-Typen beim Weizen hervorgegangen sind. — Bot. Notiser 1931: 343—345.
84. — 1932. Zytologische Studien an compactoiden Typen von *Triticum vulgare*. — Hereditas XVII: 155—196.
85. ISHIKAWA, K. 1934. Occurrence of speltoid mutants in some Japanese varieties of wheat. I—III. — Agr. and Hort. Japan 9: 1361—1371, 1556—1571, 2244—2247 (ref. Pl. Breed. Abstr. 6: 260).
86. JENKIN, T. J. 1925. Natural crossing in wheat. — Welsh Jour. Agric. 1: 104—110.
87. JUNGFER, E. 1953. Speltoid- und Compactoidmutationen. — Der Züchter 23: 273—280.
88. KAJANUS,, B. 1918. Kreuzungsstudien an Winterweizen. — Bot. Notiser 1918: 235—244.
89. — 1923. Genetische Untersuchungen an Weizen. — Bibliotheca Genetica 5: 1—187.
90. — 1924. Über eine eigenartige Ährenanomalie bei Weizen. — Hereditas V: 217—221.
91. — 1927. Die Ergebnisse der genetischen Weizenforschung. — Bibliographia Genetica 3: 141—244.
92. KATTERMANN, G. 1936/37. Genetische Ergebnisse bei Weizenroggenbastarden bis

F_4. Mitteilung II. Über anscheinend konstante Bastardformen mit behaartem Halm, ihre Abstammung und ihre Merkmalsverhältnisse. — Pfl.-bau 13: 15—45.

93. KATTERMANN, G. 1937. Chromosomenuntersuchungen bei halmbehaarten Stämmen aus Weizenroggenbastardierung. — Zschr. ind. Abst. Vererb. 73: 1—48.
94. — 1938. Das Verhalten des Chromosoms für Behaarung roggenbehaarter Nachkommen aus Weizenroggenbastardierung in neuen Kreuzungen mit Roggen und Weizen. — Zschr. ind. Abst. Vererb. 74: 1—16.
95. KIHARA, H. 1944. Discovery of the DD-analyser, one of the ancestors of *Triticum vulgare*. — Agr. and Hort. Japan 19: 889—890.
96. KIHARA, H. and LILIENFIELD, F. 1949. A new synthesized 6x-wheat. — Proc. 8th Int. Congr. Genet. (Hereditas, Suppl. Vol.): 307—319.
97. KIHARA, H. and NISHIYAMA, I. 1937. Possibility of crossing-over between semihomologous chromosomes from two different genomes. — Cytologia, FUJII Jub. Vol.: 654—666.
98. KIHARA, H., OKAMOTO, M., IKEGAMI, M., TABUSHI, J., SUEMOTO, H. and YAMANE, Y. 1950. Morphology and fertility of five new synthesized hexaploid wheats. — Seiken Zihô 4: 127—140.
99. KOLLER, P. C. 1938. Asynapsis in *Pisum sativum*. — Jour. Genet. 36: 275—306.
100. KOSTOFF, D. 1937. Formation of a quadrivalent group in a hybrid between *Triticum vulgare* and a *Tr. vulgare* extracted derivative. — Curr. Sci. 5: 537.
101. LATHOUWERS, V. 1930. La variabilité (non fluctuante) dans un lignée de *Triticum vulgare*. Apparition de variantes »speltoides» steriles. — Bull. Soc. Roy. Bot. Belgique 63: 49—54.
102. LEA, D. E. 1946. Actions of radiations on living cells. — Univ. Press, Cambridge, 402 pp.
103. LEA, D. E. and CATCHESIDE, D. G. 1942. The mechanism of the induction by radiation of chromosome aberrations in *Tradescantia*. — Jour. Genet. 44: 216—245.
104. LEIGHTY, C. E. and BOSHNAKIAN, S. 1921. Genetic behaviour of the spelt form in crosses between *Triticum Spelta* and *Triticum sativum*. — Jour. Agr. Res. 22: 335—364.
105. LEVAN, A. 1942. Studies on the meiotic mechanism of haploid rye. — Hereditas XXVIII: 177—211.
106. LEWIS, E. B. 1951. Pseudoallelism and gene evolution. — Cold Spring Harbor Symp. Quant. Biol. 16: 159—172.
107. LI, H. W., HSIA, C. A. and LEE, C. L. 1948. An inquiry into the nature of speltoid and compactoid types of wheat as a function of genic dosage. — Bot. Bull. Acad. Sinica 2: 243—264.
108. LIMA-DE-FARIA, A. 1953. Structure and properties of the kinetochore. — Proc. 9th Int. Congr. Genet. (in the press).
109. LINDHARD, E. 1922. Zur Genetik des Weizens. — Hereditas III: 1—90.
110. — 1923. Fortgesetzte Untersuchungen über Speltoidmutationen. — Hereditas IV: 206—220.
111. — 1927. Über Ährendichte und Spaltungsmodi der Speltoidheterozygoten. — Kgl. Vet. Landbohøjsk. Aarsskr., Medd. Nr. 4, 37 pp.
112. LOVE, R. M. 1940 a. A cytological deficient speltoid of hybrid origin. — Genetics 25: 126.

113. LOVE, R. M. 1940 b. Chromosome number and behaviour in a plant breeder's sample of pentaploid wheat hybrid derivatives. — Can. Jour. Res. C 18: 415—434.

114. — 1951. Varietal differences in meiotic chromosome behaviour of Brazilian wheat. — Agron. Jour. 43: 72—76.

115. MAC KEY, J. 1951. Neutron and X-ray experiments in barley. — Hereditas XXXVII: 421—464.

116. — 1952. The biological action of X-rays and fast neutrons on barley and wheat. — Arkiv Bot. S 2, Bd 1, Nr 16: 545—556.

117. — 1953. Mutability at diploid and polyploid level. — Proc. 9th Int. Congr Genet. (in the press).

118. — 1954 a. The biological action of mustards on dormant seeds of barley and wheat. — Acta Agric. Scand. 4 (in the press).

119. — 1954 b. Mutation breeding in polyploid cereals. — Acta Agric. Scand. 4 (in the press).

120. — 1954 c. X-ray sensitivity in relation to the spring and winter habit of plants. — (In preparation.)

121. MALINOWSKI, E. 1914. Les hybrides du froment. — Bull. Acad. Sci. Cracovie, Sci. Math. Nat. Ser. B: 410—450.

122. — 1916. Über die durch Kreuzung hervorgerufene Vielförmigkeit beim Weizen. — Compt. Rend. Soc. Sci. Warszawa 9: 733—756.

123. MARQUARDT, H. 1948. Das Verhalten röntgeninduzierter Viererringe mit grossen interstitiellen Segmenten bei *Oenothera Hookeri*. — Zschr. ind. Abst. Vererb. 82: 415—429.

124. MARSHAK, A. 1939. Effects of fast neutrons on chromosomes in mitosis. — Proc. Soc. Exp. Biol. Med. 41: 176—180.

125. MARSHAK, A. and BRADLEY, M. 1944. X-ray inhibition of mitosis in relation to chromosome number. — Proc. Nat. Acad. Sci. USA. 30: 231—237.

126. MATSUMURA, S. 1939. Das fehlende Chromosom beim B-Speltoidweizen. — Jap. Jour. Genet. 15: 323—324 (ref. Pl. Breed. Abstr. 10: 1011).

127. MC CLINTOCK, B. 1941. The stability of broken ends of chromosomes in *Zea mays*. — Genetics 26: 234—282.

128. MC FADDEN, E. S. 1939. Brown necrosis, a discoloration associated with rust infection in certain rust-resistant wheats. — Jour. Agr. Res. 58: 805—819.

129. MC FADDEN, E. S. and SEARS, E. R. 1944. The artificial synthesis of *Triticum Spelta*. — Records Genet. Soc. Amer. 13: 26—27.

130. — and — 1946. The origin of *Triticum spelta* and its free-threshing hexaploid relatives. — Jour. Hered. 37: 81—116.

131. MENZEL, M. Y. and BROWN, M. S. 1952. Viable deficiency-duplications from a translocation in *Gossypium hirsutum*. — Genetics 37: 673—692.

132. MICZYNSKI, K. JR. 1930. O dziedziczeniu sie niektórych cech u pszenicy w krzyzówkach *Triticum pyramidale*×*T. durum* i *T. vulgare*×*T. sphaerococcum*. — Roczn. Nauk. rol. 23: 27—62.

133. MILLER, E. C., GAUCH, H. G. and GRIES, G. A. 1944. A study of the morphological nature and physiological functions of the awns of winter wheat. — Kansas Agr. Exp. Sta., Techn. Bull. 57, 82 pp.

134. MORITZ-VOM BERG, H. 1935. Zytologische Untersuchungen an der Nachkommen-

schaft künstlich erzeugter Weizenmutanten. — Ber. deutsch. bot. Ges. 53: 548—559.

135. MYERS, W. M. and POWERS, L. 1938. Meiotic instability as an inherited character in varieties of *Triticum aestivum*. — Jour. Agr. Res. 56: 441—452.

136. MÜNTZING, A. 1930. Einige Beobachtungen über die Zytologie der Speltoidmutanten. — Bot. Notiser 1930: 35—47.

137. — 1941. Differential response to X-ray treatment of diploid and tetraploid barley. — Kungl. Fysiogr. Sällsk. i Lund Förhandl. Bd 11, Nr 6, 10 pp.

138. — 1942. Frequency of induced chlorophyll mutations in diploid and tetraploid barley. — Hereditas XXVIII: 217—221.

139. MÜNTZING, A. and PRAKKEN, R. 1941. Chromosomal aberrations in rye populations. — Hereditas XXVII: 273—308.

140. NAGAI, M. and LOCHER, G. L. 1938. The production of mutations in *Drosophila* with neutron radiation. — Genetics 23: 179—189.

141. NILSSON, E. 1934. Paralleles Auftreten von *Tilletia*-Infektion und Speltoidcharacter bei *Triticum vulgare*. — Hereditas XVIII: 262—268.

142. NILSSON-EHLE, H. 1911. Kreuzungsuntersuchungen an Hafer und Weizen. II. — Lunds Univ. Årsskr. N. F. Afd. 2, Bd 7, Nr. 6, 84 pp.

143. — 1915. Gibt es erbliche Weizenrassen mit mehr oder weniger vollständiger Selbstbefruchtung? — Zschr. Pfl.-zücht. 3: 1—6.

144. — 1917. Untersuchungen über Speltoidmutationen beim Weizen. — Bot. Notiser 1917: 305—329.

145. — 1920. Multiple Allelomorphe und Komplexmutationen beim Weizen. — Hereditas I: 277—311.

146 — 1921. Über mutmassliche partielle Heterogamie bei den Speltoidmutationen des Weizens. — Hereditas II: 25—76.

147. — 1927. Das Verhalten partieller Speltoidmutationen bei Kreuzung untereinander. — Hereditas IX: 369—379.

148. NILSSON-LEISSNER, G. 1925. Beiträge zur Genetik von *Triticum Spelta* und *Triticum vulgare*. I. — Hereditas VII: 1—74.

149. NISHINA, Y. and MORIWAKI, D. 1939. Induction of sex-linked lethals in *Drosophila* by neutrons. — Sci. Pap. Inst. Phys. Chem. Tokyo 36: 419—425.

150. — and — 1941. Induction of sex-linked lethals in *Drosophila* by neutrons. — Sci. Pap. Inst. Phys. Chem. Tokyo 38: 371—376.

151. NISHIYAMA, I. 1933. On the meachanism of the fatuoid and speltoid mutation. — Kwagaku Japan 3: 147—152.

152. NORONHA WAGNER, M. 1950. The action of X-rays on chromosomes of *Triticum vulgare* HOST. — Genet. Iberica 2: 39—52.

153. NYBOM, N., GUSTAFSSON, Å. and EHRENBERG, L. 1952. On the injurious action of ionizing radiations in plants. — Bot. Notiser 1952: 343—365.

154. OEHLER, E. 1930. Speltoid- und Fatuoidmutationen. — Der Züchter 2: 93—101.

155. OLTMANN, W. 1950. Züchterische Auswertung röntgeninduzierter Mutationen an physiologischen Merkmalen bei Winterweizen. — Zschr. Pfl.-zücht. 29: 76—89.

156. ONUFRIJCHUK, T. 1953. The production of speltoid mutants in spring wheat by X-irradiation. — Winnipeg, Canada, 20 pp.

157. PERCIVAL, J. 1921. The wheat plant. A monograph. — Duckworth & Co., London, 463 pp.

158. PHILIPTSCHENKO, J. 1929. Ein neuer Fall von Speltoidmutationen beim Weizen. — Zschr. ind. Abst. Vererb. 52: 106—113.

159. — 1934. Genetics of soft wheats. — State Press Lit. State Coll. Farms, Moscow-Leningrad, 262 pp.

160. PHIPPS, I. F. and GURNEY, H. C. 1932. A preliminary note on the origin of a B-type speltoid in *Triticum vulgare*. — Austral. Jour. Exp. Biol. Med. Sci. 10: 215—218.

161. PIROVANO, A. 1939. Stimoli mutativi sul grano. — Ricerca Sci. 10: 693—703.

162. POWERS, L. 1932 a. Cytologic and genetic studies of variability of strains of wheat derived from interspecific crosses. — Jour. Agr. Res. 44: 797—831.

163. — 1932 b. Cytological aberrations in relation to wheat improvement. — Jour. Amer. Soc. Agron. 24: 531—536.

164. — 1932 c. Cytological aberrations in *Triticum*. — Proc. 6th Int. Congr. Genet. Ithaca, N. Y. 2: 160—161.

165. RANJAN, S. 1940. A preliminary note on the X-ray mutants of Pusa (52) wheat. — Proc. Indian Acad. Sci. B 12: 62—66.

166. — 1946. Studies on the Ranjan X-ray mutant wheats. I. Genetical analysis of the eleven variants. — Proc. Nat. Acad. Sci. India B 16: 287—292.

167. RAUM, H. 1931. Über die Vererbung von Ähreneigenschaften bei Kreuzungen zwischen Emmer- und Dinkelreihe des Weizens. — Zschr. Pfl.-zücht. 16: 161—254.

168. RÖSLER, P. 1928. Histologische Studien am Vegetationspunkt von *Triticum vulgare*. — Planta 5: 28—69.

169. SANCHEZ-MONGE, E. 1949. Note on meiotic bridges in *Triticum*. — Genet. Iberica 1: 55—57.

170. — 1950 a. Two types of misdivision of the centromere. — Nature 165: 80.

171. — 1950 b. Univalent mechanism and misdivision. — Anal. Est. Exp. Aula Dei 2: 1—11.

172. — 1951. The stability of isochromosomes. — Anal. Est. Exp. Aula Dei 2: 168—173.

173. SANCHEZ-MONGE, E. and MAC KEY, J. 1948. On the origin of subcompactoids in *Triticum vulgare*. — Hereditas XXXIV: 321—337.

174. SAPEHIN, A. A. 1928. Hylogenetic investigations of the *vulgare* group in *Triticum*. — Bull. Appl. Bot. Genet. Pl. Breed. 19: 127—166.

175. — 1930. Röntgen-Mutationen beim Weizen (*Triticum vulgare*). — Der Züchter 2: 257—259.

176. — 1934. X-ray mutations as a source of new varieties of agricultural plants. — Priroda 9: 28—31 (ref. Pl. Breed. Abstr. 5: 274).

177. — 1935. X-ray mutants in soft wheat. — Bull. Appl. Bot. Genet. Pl. Breed. Ser. 2. 9: 3—37.

178. SCHIEMANN, E. 1932. Pfahlbauweizen. Historisches und Phylogenetisches. — Zschr. Pfl.-zücht. 17: 36—54.

179. — 1947. Über MC FADDEN—SEARS's Theorie zur Phylogenie des Weizens. — Der Züchter 17/18: 385—391.

180. — 1948. Weizen, Roggen, Gerste. Systematik, Geschichte und Verwendung. — Verlag Gustav Fischer, Jena, 102 pp.

181. — 1949. Die neue Nomenklatur der Getreidearten. — Der Züchter 19: 322—325.

182. SCHKWARNIKOW, P. K. 1936. Einfluss hoher Temperatur auf die Mutationsrate bei Weizen. — Planta 25: 471—480.
183. — 1937. Über Erhöhung der Mutationsrate bei Weizen nach langer Aufbewahrung der Samen. — Genetica 19: 188—199.
184. — 1939. Mutation in seeds and its significance for a seed culture and selection of plants. — Bull. Acad. Sci. URSS. 1939: 1009—1054.
185. SCHRIMPF, K. 1951. Ein Beitrag zur Phylogenie und Systematik der Gattung *Triticum*. — Zschr. Pfl.-zücht. 31: 42—71.
186. SEARS, E. R. 1944. Cytogenetic studies with polyploid species of wheat. II. Additional chromosomal aberrations in *Triticum vulgare*. — Genetics 29: 232—246.
187. — 1946. Isochromosomes and telocentrics in *Triticum vulgare*. — Genetics 31: 229—230.
188. — 1948. The cytology and genetics of the wheats and their relatives. — Adv. Genet. 2: 239—270.
189. — 1952 a. Misdivision of univalents in common wheat. — Chromosoma 4: 535—550.
190. — 1952 b. The behavior of isochromosomes and telocentrics in wheat. — Chromosoma 4: 551—562.
191. SEARS, E. R. y CAMARA, A. 1950. Un chromosoma dicentrico transmisible en trigo. — Genet. Iberica 2: 239—256.
192. — and — 1952. A transmissible dicentric chromosome. — Genetics 37: 125—135.
193. SHARMAN, B. C. 1945. Leaf and bud initiation in the *Gramineae*. — Bot. Gaz. 106: 269—289.
194. SMITH, L. 1946. A comparison of the effects of heat and X-rays on dormant seeds of cereals, with special reference to polyploidy. — Jour. Agr. Res. 73: 137—158.
195. — 1948. A haplo-viable deficiency-duplication from an interchange in *Triticum monococcum*. — Bot. Gaz. 109: 258—268.
196. — 1950. Effects of atomic bomb radiations and X-rays on seeds of cereals. — Jour. Hered. 41: 125—130.
197. SMITH, S. G. 1951. Mutations in polyploid cereals. IV. A segmental chromosome deficiency determining an α series ratio in a speltoid wheat. — Can. Jour. Bot. C 29: 607—630.
198. SMITH, S. G., HUSKINS, C. L. and SANDER, G. F. 1949. Mutations in polyploid cereals. II. The cytogenetics of speltoid wheats. — Can. Jour. Res. C 27: 348—393.
199. —, — and — 1950. Mutations in polyploid cereals. III. The cytogenetics of compactoid wheats. — Can. Jour. Res. C 28: 153—182.
200. SMITH, W. K. and HARRINGTON, J. B. 1929. Wheat albinos. — Jour. Hered. 20: 19—22.
201. STADLER, L. J. 1929. Chromosome number and the mutation rate in *Avena* and *Triticum*. — Proc. Nat. Acad. Sci. USA. 15: 876—881.
202. — 1930. Some genetic effects of X-rays in plants. — Jour. Hered. 21: 2—19.
203. — 1931. The experimental modification of heredity in crop plants. II. Induced mutations. — Sci. Agr. 11: 645—661.
204. — 1932. On the genetic nature of induced mutations in plants. — Proc. 6th Int. Congr. Genet. Ithaca, N. Y. 1: 274—294.

205. STEBBINS, G. L. 1950. Variation and evolution in plants. — Columbia Univ. Press, New York, 643 pp.

206. STUBBE, H. and BANDLOW, G. 1947. Mutationsversuche an Kulturpflanzen. I. Röntgenbestrahlungen von Winter- und Sommergersten. — Der Züchter 17/18: 365—374.

207. SUNESON, C. A., BAYLES, B. B. and FIFIELD, C. C. 1948. Effects of awns on yield and market qualities of wheat. — US. Dept. Agr. Circ. No. 783, 8 pp.

208. THODAY, J. M. 1942. The effects of ionizing radiations on the chromosomes of *Tradescantia bracteata*. A comparison between neutrons and X-rays. — Jour. Genet. 43: 189—210.

209. THOMPSON, W. P. and ROBERTSON, H. T. 1930. Cytological irregularities in hybrids between species of wheat with the same chromosome number. — Cytologia 1: 252—262.

210. TIMOFÉEFF-RESSOVSKY, N. W. und ZIMMER, K. G. 1938. Neutronenbestrahlungsversuche zur Mutationsauslösung an *Drosophila melanogaster*. — Die Naturwiss. 26: 362—365.

211. UCHIKAWA, I. 1934. Genetische-cytologische Studien an Weizenspeltoiden. I. Speltoide der C-Serie. — Botany and Zoology Japan 2: 851—864.

212. — 1936. Cytogenetic studies on speltoid wheat. — Jap. Jour. Genet. 12: 53—56 (ref. Pl. Breed. Abstr. 6: 1211).

213. — 1937. Cytogenetic studies on compactoid wheat. — Jap. Jour. Genet. 13: 9—15 (ref. Pl. Breed. Abstr. 8: 113).

214. — 1938. Cytogenetic studies on short-normal type and dwarf-compactum type in compactoid wheat. — Jap. Jour. Genet. 14: 264—267 (ref. Pl. Breed. Abstr. 9: 1444).

215. — 1939. Cytogenetic studies on dwarf-compactoid wheat with 42 chromosomes. — Jap. Jour. Genet. 15: 315—317 (ref. Pl. Breed. Abstr. 11: 112).

216. — 1941. Genetic and cytological studies of speltoid wheat. II. Origin of speltoid wheat. — Mem. Coll. Agr. Kyoto Imp. Univ. No. 50, 64 pp.

217. — 1942 a. Chromosome location of the speltoid- and the compactoid-gene in *Triticum vulgare* VILL. — Jap. Jour. Genet. 18: 123—125.

218. — 1942 b. Genetic and cytological studies of speltoid wheat. III. Origin of compactoid wheat. — Mem. Coll. Agr. Kyoto Imp. Univ. No. 52, 49 pp.

219. — 1943 a. Genetic and cytological studies of speltoid wheat. IV. Origin of dwarf-compactoid wheat. — Mem. Coll. Agr. Kyoto Imp. Univ. No. 55(1), 38 pp.

220. — 1943 b. Genetic and cytological studies of speltoid wheat. V. Origin of short-normal wheat. — Mem. Coll. Agr. Kyoto Imp. Univ. No. 55(2), 44 pp.

221. — 1946. The chromosome constitution of A type speltoid wheat. — Jap. Jour. Genet. 21: 107—180 (ref. Pl. Breed. Abstr. 22: 255).

222. — 1950. Genetic and cytological studies of speltoid wheat. VI. Chromosome location of the speltoid and the compactoid gene in *Triticum vulgare* VILL., and the position of the C-chromosome in genome. — Mem. Ehime Univ. Sect. 2 1: 1—19.

223. UNRAU, J. 1950. The use of monosomes and nullisomes in cytogenetic studies of common wheat. — Sci. Agr. 30: 66—89.

224. UNRAU, J., SMITH, W. E. and MC GINNIS, R. C. 1950. Spike density, speltoidy, and compactoidy in hexaploid wheat. — Can. Jour. Res. C 28: 273—276.

225. VASILJEV, B. 1929. On the cytology of speltoids. — Bull. Bur. Genet. Leningrad 7: 31—39.
226. VASILJEV, B. and KAMENIK, I. A. 1935. On the genetics of speltoids. — Bull. Inst. Genet. USSR 10: 7—17.
227. VAVILOV, N. I. 1923. A contribution to the classification of soft wheats. — Bull. Appl. Bot. Pl. Breed. 13: 149—257.
228. VAVILOV, N. and JAKUSHKINA, O. 1925. A contribution to the phylogenesis of wheat and the inter-species hybridisation in wheats. — Bull. Appl. Bot. Pl. Breed. 15: 3—159.
229. VAVILOV, N. I. and KOUZNETSOVA, E. S., 1921. On the genetic nature of winter and spring varieties of plants. — Ann. Agr. Fac. Saratov Univ. 1: 1—25.
230. VERVELDE, G. H. 1946. On the role of awns in cereals. — Meded. Landb. Hoogesch. Wageningen 48: 35—60.
231. VESTERGAARD, A. B. 1919. Iagttagelser vedrørende Arvelighedsforhold hos Lupin, Hvede og Byg. — Tidsskr. Planteavl 26: 491—510.
232. — 1921. Beobachtungen vom Zuchtgarten. — Zschr. Pfl.-zücht. 8: 192—195.
233. WALTERS, M. S. 1950. Spontaneous breakage and reunion of meiotic chromosomes in the hybrid *Bromus Trinii*×*B. maritimus*. — Genetics 35: 11—37.
234. — 1952. Spontaneous chromosome breakage and atypical chromosome movement in meiosis of the hybrid *Bromus marginatus*×*B. pseudolaevipes*. — Genetics 37: 8—25.
235. WATKINS, A. E. 1927. Genetic and cytological studies in wheat. IV. — Jour. Genet. 19: 81—96.
236. — 1928. The genetics of wheat species crosses. I. — Jour. Genet. 20: 1—27.
237. — 1930. The wheat species: a critique. — Jour. Genet. 23: 173—263.
238. — 1940. The inheritance of glume shape in *Triticum*. — Jour. Genet. 39: 249—264.
239. WATKINS, A. E. and ELLERTON, S. 1940. Variation and genetics of the awn in *Triticum*. — Jour. Genet. 40: 243—270.
240. WEISS, J. 1944. Radiochemistry of aequos solutions. — Nature 153: 748—750.
241. WINGE, Ö. 1924. Zytologische Untersuchungen über speltoide und andere mutantenähnliche Aberranten beim Weizen. — Hereditas V: 241—286.
242. ZHUKOVSKY, P. M. 1923. »Persian wheat» — *Triticum persicum* VAV. in Transcaucasia. — Bull. Appl. Bot. Pl. Breed. 13: 45—55.
243. ZIMMER, K. G. und TIMOFÉEFF-RESSOVSKY, N. W. 1938. Dosimetrische und strahlenbiologische Versuche mit schnellen Neutronen II. — Strahlentherapie 63: 528—536.
244. ÅKERMAN, Å. 1920. Speltlike bud-sports in common wheat. — Hereditas I: 116—127.
245. — 1922. Untersuchungen über eine in direktem Sonnenlichte nicht lebensfähige Sippe von *Avena sativa*. — Hereditas III: 147—177.
246. — 1923. Beiträge zur Kenntnis der Speltoidmutationen des Weizens. I. Untersuchungen über eine Speltoidform aus schwedischem Sammetweizen. — Hereditas IV: 111—124.
247. — 1927. Weitere Studien über Speltoidchimären bei *Triticum vulgare*. — Hereditas IX: 321—334.
248. — 1929. Letalfaktorer hos havre och vete. — Beretn. N. J. F. 4. Kongr. Helsingfors: 594—604.

249. Åkerman, Å. 1943. Försök till stegrande av vårvetets avkastning. I. Korsningar med Brunt Schlanstedtervårvete jämte beskrivning av Svalöfs Progressvårvete. — Sveriges Utsädesf. tidskr. 53: 51—66.
250. — 1944. Svalöfs Skandiavete III. — Sveriges Utsädesf. tidskr. 54: 63—65.
251. — 1951. Svalöfs Rivalvårvete (01029 b). — Sveriges Utsädesf. tidskr. 61: 45—49.
252. Åkerman, Å. und Fröier, K. 1941. Studien über eine spontane *Chlorina*-Mutation in *Avena sativa*. — Hereditas XXVII: 371—404.
253. Åkerman, Å. and Mac Key, J. 1948. A genetical analysis of some speltoid strains. — Hereditas XXXIV: 301—320.
254. — och — 1949. Försök till stegrande av vårvetets avkastning. II. Korsningar mellan vår- och höstvete. Beskrivning av Svalöfs Ellavårvete. — Sveriges Utsädesf. tidskr. 59: 105—117.

CONTENTS.

I. Introduction 65
II. Material and methods 68
III. Effects of radiation in the first generation 70
1. The differential response to X-rays and fast neutrons 70
2. Morphological abnormalities in the first generation 74
IV. Rates of mutations observed in the second generation 80
1. The spontaneous frequency of mutations in 6*x*-wheat 80
2. The frequency of induced mutations in 6*x*-wheat. 88
V. Analysis of the induced mutations 97
1. Speltoid mutations 98
A. Speltoid mutations of deficiency-type 104
B. Speltoid mutations of monosome-type 124
C. The interrelation of deficiency- and monosome-speltoids of different origins 127
2. Dense-eared mutations 132
3. Lax-eared mutations 142
4. Bearded mutations 146
5. Short-strawed mutations 148
6. Chlorophyll mutations 149
7. Other mutations 149
VI. Discussion 152
Acknowledgments 166
Summary 167
Literature cited 169

Communications from the Swedish Seed Association. No. 73.

FERTILITY AND ANEUPLOIDY IN NEW AUTOTETRAPLOIDS IN LAMIUM

BY *PETER BERNSTRÖM*
INSTITUTE OF GENETICS, LUND, SWEDEN

I. INTRODUCTION.

FOR the purposes of an investigation into the relationships between various *Lamium* species native to Northern Europe, autotetraploids of the two species *L. amplexicaule* L. and *L. purpureum* L. have been produced. The aims of the investigation and the reason for producing the autotetraploids have previously been stated (BERNSTRÖM, 1949, 1953 a). A full presentation of these species and of their characters has also been given (BERNSTRÖM, 1952, 1953 b).

While the relationships in *Lamium* were being studied, and not least in order to provide supplementary data for that investigation, another investigation into the characters of the autotetraploids of *L. amplexicaule* and *L. purpureum* was in progress. This paper will present the results of the latter study. The effects of chromosome number doubling on the fertility of cleistogamous and chasmogamous flowers have been published elsewhere (BERNSTRÖM, 1950 b). The autotetraploids were chiefly produced for use in hybridization experiments, however, and their study was therefore occasionally allowed to yield precedence to projects of greater immediate importance owing to lack of space, time, labour, *etc.* As a result, these studies were not always as complete as one might have wished.

Roughly speaking, the present investigation had a twofold aim. The first was to estimate the degree of fertility of the tetraploids and compare it with those of the diploids and allopolyploids. Simultaneously, attempts were made to improve the fertility of the autotetraploids. This was done partly because it would *per se* be interesting to know whether such improvement can be brought about, partly because a maximal fertility would seem to be of advantage in hybridization experiments — particularly when the desired combinations are difficult to attain — and for studies of species hybrids' fertility which might be dependent on the fertility of the parents. The second objective of the investigation was to ascertain the variability of the chromosome number in the autotetraploids and to estimate by how much repeated selection of the ex-

treme aneuploids could extend its range of variation. Since widely scattered chromosome numbers had been found in the F_2 progeny of interspecific *Lamium* hybrids, it would be interesting to contrast this variability and its manifestations and the corresponding phenomena in the pure species. However, that will be the subject of a forthcoming paper. Here merely the intraspecific range of chromosome number variation and its effects on various characters will be dealt with.

II. MATERIALS, METHODS AND GENERAL CHARACTERS OF THE AUTOTETRAPLOIDS.

The autotetraploids of *L. amplexicaule* and *L. purpureum* used experimentally were all derived from lines which for the most part were identical with those used for the experimental studies presented in preceding papers (BERNSTRÖM, 1952, 1953 b), which may be consulted for data on the line characters.

The following lines were used:

Of tetraploid *L. amplexicaule*

*a*14. Derived from line *a*8, habitat: Gothenburg, Sweden.
*a*15. Derived from line *a*9, habitat: Svalöf, Sweden.
*a*16. Derived from a line with the same habitat as *a*8 and resembling it so closely that the two lines probably were identical genetically; *a*16's first ancestral line soon became extinct.
*a*17. Derived from line *a*10, habitat: Svalöf.
*a*27. Derived from line *a*12, habitat: Coimbra, Portugal.
*a*28. Derived from line *a*13, habitat: Bucharest, Roumania.
*a*55. Derived from line *a*32, habitat: Helsingborg, Sweden.

Of tetraploid *L. purpureum*

*p*6. Derived from line *p*1, habitat: Gothenburg, Sweden.
*p*7. Derived from line *p*2, habitat: Gothenburg.
*p*30. Derived from line *p*25, habitat: Lund, Sweden.
*p*42. Derived from line *p*26, habitat: Waadt, Switzerland.
*p*43. Derived from line *p*29, habitat: Malmö, Sweden.
*p*59. Derived from line *p*51, habitat: Lund.
*p*60. Derived from line *p*53, habitat: Lund.
*p*61. Derived from line *p*54, habitat: Lund.
*p*62. Derived from a diploid line (habitat: Lund) characterized by unpatterned unveined flowers coloured evenly red.

Most of the tetraploids were produced by applying to the growing point of the shoots and to the leaf axils of very young seedlings a 1,6 per cent colchicine solution in a 1 per cent suspension of agar-agar in the solid or liquid state. Those branches were saved whose flowers and general appearance indicated that the chromosome number had doubled. Among the offspring those plants were selected whose general appearance and seed setting showed that the same change had occurred. This method of producing the desired tetraploids, as a rule, met with no difficulties. That the chromosome number actually had doubled was later checked by cytological examination. Offspring of the tetraploid branches produced by colchicine treatment were assigned the symbol C_1 (1st tetraploid generation), their offspring C_2, *etc.*

The cytological methods adopted have previously been described (BERNSTRÖM, 1952). Most of the root tip fixations (in modified Navashin's solution) were very successful. Usually chromosome number determinations were done in three metaphase plates with three root tips from each plant. If the first three plates yielded discrepant chromosome counts, sufficient extra plates were counted to provide an adequate preponderance for one of the numbers obtained. In doubtful cases fresh preparations were made.

The tetraploid lines were consistently cultivated from seeds from the plant or plants having the highest seed fertility in the preceding generation. This was done to reduce to a minimum the risk of using seeds from aneuploids either for studying the properties of the autotetraploids or in hybridization experiments. Seed setting is, as we shall see, the character whereby euploids most readily can be distinguished from aneuploids.

Reference is made to a preceding publication (BERNSTRÖM, 1952) for further notes on methods.

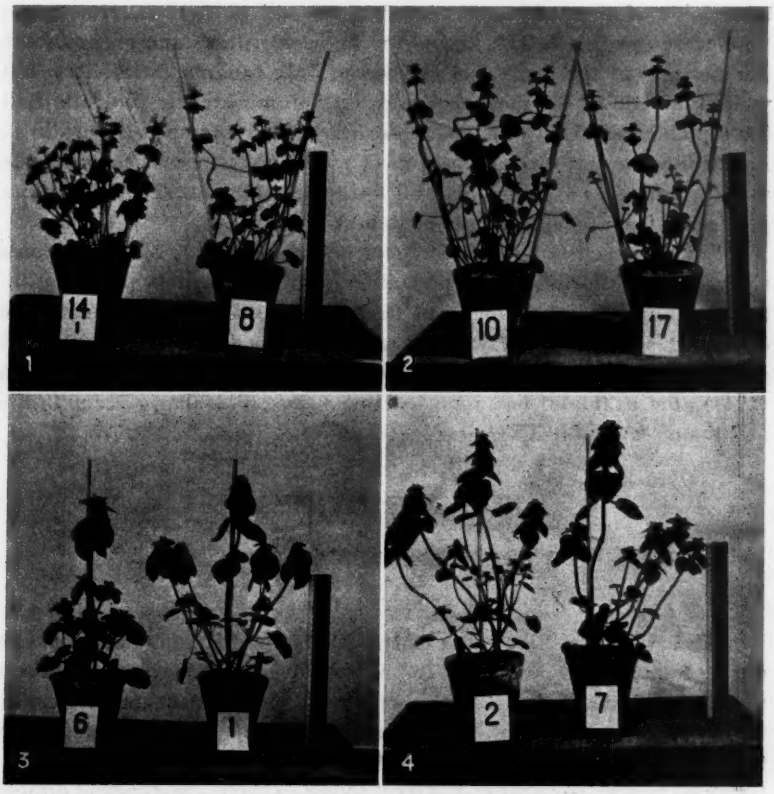
The autotetraploids of *L. amplexicaule* and *L. purpureum* are, like the diploids, self-fertilizing and consequently they form pure lines. The flower biology of tetraploid *L. amplexicaule* has already been treated at length (BERNSTRÖM, 1950 b), when it was shown that, among other things, the chasmogamous flowers had a significantly lower self-fertilizing power than the diploids which themselves had a somewhat impaired self-fertilizing power. Nevertheless, and open flowering notwithstanding, not a single case of vicinism was observed among the cultivated individuals of tetraploid *L. amplexicaule*.

The tetraploid *L. purpureum* resembles the diploid in having self-pollinating flowers (*cf.* BERNSTRÖM, 1950 a, 1952) which fertilized them-

selves far more readily than in tetraploid *L. amplexicaule*, the proportion of unpollinated flowers on plants under glass and inaccessible to insects not exceeding 5 per cent. In the diploids this percentage is negligibly lower. On the other hand the diploid and even more so the tetraploid *L. purpureum* are much sought after by nectarivorous Hymenoptera (*cf.* BERNSTRÖM, 1952). As a consequence at least six and probably a few more cases of vicinism occurred: two were hybrids with *L. hybridum* and have previously been described (*loc. cit.*), and three were directly observed intraspecific hybrids within two lines which occurred in different years. Some outcrossings probably occurred in the C_1 generation of *p*30, later altering the appearance of the line and improving the seed setting. We shall return to this question in due course.

The tetraploids of *L. amplexicaule* and *L. purpureum* retain their specific characters well. However, owing to the coarser organs exhibited by both species after doubling of the chromosome complement, it was an easy matter to distinguish the tetraploids from the diploids. The pure lines remained at least as characteristic as tetraploids as they had been as diploids, for the typical line features were unchanged or even accentuated in various ways in the tetraploids, in all probability by virtue of the aforementioned coarsening. This was manifested in a number of ways, such as larger flowers with stronger hues, larger and less abundant teeth on the leaves, stiffer leaves of slightly altered shape and darker hue, larger calyces, *etc.* Particularly in tetraploid *L. purpureum* the tissues often acquire a slightly disharmonious macroscopical appearance. For example, the stem frequently presents a wrinkled skin, the leaves tend to become dished, the nerve knots on the biggest leaves very often become disproportionately large, and so on. As another manifestation of the same thing one might consider the fact that the three or four lowest axils on the stem, which in diploids practically always carry well developed branches, very often in tetraploids give origin to no branches whatsoever. This brings to mind the common observation that tetraploids have fewer tillers than diploids (*cf.* SCHWANITZ and SCHWANITZ, 1950; PIRSCHLE, 1942; MÜNTZING, 1951; *etc.*).

While the tetraploids in *L. amplexicaule* become no taller than the diploids, they usually do so, if only a little, in *L. purpureum*. On the other hand the tetraploids in both species generally have slightly shorter branches than the diploids. In summertime flowering begins more or less at the same time in the tetraploids and the diploids; in late summer generations the tetraploids tend to bloom before the diploids in *L. amplexicaule* and after the diploids in *L. purpureum*. The tetraploids in



Figs. 1—4. Diploids and the corresponding tetraploids of *L. amplexicaule* (Figs. 1—2) and of *L. purpureum* (Figs. 3—4). — The numbers in the Figs. are line numbers without the appropriate letters. The ruler was 30 cm long.

both species survive longer, however, the difference ranging between one and about three weeks depending on the season. Moreover, the tetraploid flowers stay longer in the calyces and wither more slowly. Depicting a diploid and a tetraploid from two lines of each species, Figs. 1 to 4 are intended to provide an idea of the habitual appearance of the material.

The pollen grains, nearly spherical in both species, were used to determine the difference between the cell volumes of diploids and tetraploids. Measurements were made on pollen stored in a mixture of equal parts of aceto-carmine and glycerol. Pollen grains from diploids and

tetraploids of three *L. amplexicaule* lines and of four *L. purpureum* lines were measured. The means for lines with the same chromosome number and of the same species showed only random differences; but while the ratio of the mean pollen grain volume for tetraploids to that for diploids was 2,07 : 1 in *L. amplexicaule*, the corresponding ratio in *L. purpureum* was 1,79 : 1. An almost identical difference between the average cell sizes for diploids and tetraploids was obtained in *L. amplexicaule* from measurements of stomata.

Since the cell volume differences between diploids and tetraploids showed good interlinear and poor interspecific agreement, it is reasonable to suppose that doubling of the chromosome number might have dissimilar sequels in the two species, at least as far as inclining pollen grain volume goes. Such divergences are not uncommon. VON WOESS (1941), for example, has reported one between *Arenaria serpyllifolia* and the closely related species *A. Marschlinsii*.

III. EXPERIMENTAL.

1. L. AMPLEXICAULE.

A. FERTILITY.

The pollen fertility was examined in four different lines through one to four generations. Between six and fifty-four plants were studied per line and generation, the proportion of good pollen grains ranging from 85,6 to 93,4 per cent. No line was consistently inferior or superior to the other lines. The pollen fertility of the tetraploid lines ranged between 87 and 95 per cent of the level found in the corresponding diploid lines.

The seed setting recorded in pure lines of tetraploid *L. amplexicaule* is given in Table 1. It will be noted that, ostensibly at least, the seed setting underwent a far greater change for the worse after the transformation from diploid to tetraploid state than the pollen fertility did. The means varied widely from one line to another of the summer generations and within fairly narrow limits in the autumn generations, *i. e.* in much the same way as in the diploids (*cf.* BERNSTRÖM, 1952). From line to line and generation to generation the diploid means stood in a fairly constant ratio to the tetraploid means; low diploid fertility was thus accompanied by low tetraploid fertility in the same line. In generations 44—1 and 45—1, *a*14 (+*a*16) and *a*15 exhibited relatively sharper declines than other lines. Certainly for *a*14 and prob-

TABLE 1. *Seed setting in pure lines of tetraploid L. amplexicaule.*

Generations	44—1				44—2				45—1				45—2				46			
Lines	$\bar{x}$	in % of the diploid	n	C-generation	$\bar{x}$	in % of the diploid	n	C-generation	$\bar{x}$	in % of the diploid	n	C-generation	$\bar{x}$	in % of the diploid	n	C-generation	$\bar{x}$	in % of the diploid	n	C-generation
*a*14	$39{,}2 \pm 2{,}17$	49	34	C3	$50{,}1 \pm 1{,}55$	51	31	C4	$30{,}7 \pm 1{,}68$	42	35	C5	$52{,}8 \pm 2{,}25$	59	19	C6	$51{,}7 \pm 2{,}24$	61	17	C7
*a*15	$32{,}5 \pm 2{,}22$	35	35	C3	$48{,}4 \pm 1{,}98$	50	18	C4	$39{,}2 \pm 1{,}70$	44	50	C5	$46{,}8 \pm 2{,}17$	50	15	C6	$40{,}9 \pm 3{,}62$	—	12	C6
*a*16	$39{,}7 \pm 1{,}21$	49[1]	47	C6					$32{,}6 \pm 1{,}35$	45[1]	22	C7	$53{,}4 \pm 2{,}97$	59[1]	12	C8				
*a*17	$56{,}1 \pm 1{,}88$	57	40	C6	$49{,}4 \pm 2{,}60$	50	39	C7	$60{,}4 \pm 1{,}54$	64	43	C8	$52{,}5 \pm 1{,}98$	55	20	C9				
*a*27									$50{,}0 \pm 1{,}61$	64	21	C4	$52{,}8 \pm 2{,}31$	58	11	C5				
*a*28									$51{,}4 \pm 3{,}03$	—	22	C3	$45{,}8 \pm 3{,}12$	—	14	C4	$57{,}5 \pm 2{,}17$	62	11	C5
*a*55													$55{,}7 \pm 1{,}72$	61	14	C2	$60{,}5 \pm 3{,}12$	66	11	C3

[1] Computed on the mean of *a*8.

ably for *a*15 this finds an explanation in the reduced seed-setting power of chasmogamous flowers as compared with cleistogamous flowers which has been reported already (BERNSTRÖM, 1950 b). In *a*14, *a*16 and probably *a*15 the summer generations of 1944 and 1945 showed marked seed-setting differences compared with the corresponding autumn generations because there was practically no chasmogamous flowering in the autumn of both years — the differences from generation to generation are in all cases significant. The 1946 mean for *a*14 was, however, practically the same as the 1945—2 mean and differed but little from the 1947 mean ($46{,}1 \pm 2{,}86$, *i. e.* the only mean not included in the table).

The causative factor here cannot have been subnormal chasmogamous flowering, because this was about as profuse in 1946 and 1947 as during preceding summer generations. Nor can outcrossing have improved the fertility of *a*14, for that would have been immediately reflected in the appearance of the plants. The most probable, though not completely satisfying, explanation for the better seed setting in 1946 and 1947 compared to preceding summers is that the greenhouse in these years was insulated against insects, reducing the air circulation and raising the temperature. Moreover, at least 1947 was itself a very hot year. As will be shown, a high temperature would seem to improve the seed setting in the cleistogamous flowers, which might have counteracted the unfavourable effect of the chasmogamous flowers on the seed setting. Yet this is not borne out by experiences reported in a previous paper (BERNSTRÖM, 1950 b).

Thus, while the seasonal seed-setting variation of *a*14 (with *a*16) and *a*15 under normal temperature conditions displayed a higher degree of the same tendency as their diploid progenitors, a different if not opposite tendency was observed in *a*17. In the years 1944 and 1945 the summer and autumn seed setting of *a*10 were equally good, whereas the summer seed setting of *a*17 exceeded its autumn seed setting, sometimes significantly (BERNSTRÖM, 1952). *a*28 displayed a tendency resembling that of *a*17. *a*17, *a*28 and also *a*55 have in common the almost complete absence of chasmogamous flowers in the lowest whorl and, compared with other lines, the paucity of such flowers in the lowest whorl but one. Considering that the two bottom whorls were those used in determining the seed fertility, the main reason for the better seed setting during the summer than during the autumn in *a*17, *a*28 and perhaps also *a*55 would seem to be the fact that even the summer seed fertility of these lines was largely determined by the superabundance of cleistogamous flowers. In other words, for these three lines the summer would

provide conditions more amenable to seed setting than the autumn. With respect to the proportion of chasmogamous flowers in the two lower whorls, *a*15 occupied a position intermediate between those of *a*14 and *a*17, and therefore it is not quite clear why *a*15's seed setting varied as it did.

It is rather surprising that conditions more propitious for the seed setting in the cleistogamous flowers of some lines at least of tetraploid *L. amplexicaule* obtained during the warmer season. For in the rest of the *Lamium* materials studied the prevailing rule seemed to be that the seed fertility was as good in the summer as in the autumn. Thus, presuming that this conclusion is warranted, we find that the tetraploids differed from the diploids in having other ecological requirements for, or being more susceptible to, cleistogamous flowering. It should be noted, however, that, as stated above, a study of the cleistogamous seed setting in *a*14 has disclosed no such alteration (BERNSTRÖM, 1950 b).

The seed setting in interlinear hybrids of tetraploid *L. amplexicaule* has already been dealt with in another context (BERNSTRÖM, 1952). It was then demonstrated that of four hybrid combinations studied two — *a*14×*a*17 and *a*14×*a*28 — had a seed fertility whose superiority over the most fertile of the corresponding parent lines was significant and fairly significant, respectively. Thus the mean for F_1 of *a*14×*a*17/44—2 was $62{,}3 \pm 2{,}49$ (n=8) and for F_1 of *a*14×*a*28/45—2 it turned out to be $62{,}8 \pm 4{,}96$ (n=5). The latter mean for the same generation of *a*14 makes t equal to 1,83 ($0{,}1 > P > 0{,}05$). Similarly, the seed fertility of F_2 and F_3 of *a*14×*a*28 exceeded the most fertile parent line's, indicating that the seed fertility of the hybrids showed a real superiority. For F_2/45—1 the mean was $60{,}5 \pm 1{,}83$ (n=12), which with *a*28/45—1 yields $t=2{,}57$ ($0{,}02 > P > 0{,}01$); and for F_3/45—2 the mean worked out at $60{,}0 \pm 2{,}66$ (n=12), which in combination with *a*14/45—2 gives $t=2{,}07$ ($P \simeq 0{,}05$). The anomaly that F_2 was produced during the first generation of 1945 while F_1 was produced during the second is due to the fact that the F_2 and F_3 generations were derived from a single F_1 plant. Additional F_1 hybrids were produced to be on the safe side, but as the parents constituted pure lines the inverted sequence of the generations should not have affected the results. F_2 of *a*14×*a*17 would have been studied too, had not all the specimens been accidentally destroyed.

Furthermore, hybrids between *a*14 and *a*15 (1945—2) as well as between *a*14 and *a*27 (1945—1) were studied, but their seed fertility proved no better than that of the parent lines, the respective means being $47{,}3 \pm 6{,}37$ (n=4) and $41{,}1 \pm 3{,}12$ (n=11). Since *a*14×*a*27 was

studied in the summer when chasmogamous flowers were plentiful and the measure of the hybrids' true seed-setting power which, as we have seen, is attributable chiefly to the cleistogamous flowers consequently was rather unreliable, an F_2 generation was studied during the autumn of 1945. However, its seed setting was also intermediate between those of the parent lines, the mean being $51{,}4 \pm 3{,}19$ (n=13). The fact that all the hybrid generations of $a14 \times a28$ except F_2 were observed during the autumn when cleistogamous flowers were preponderant makes the result more reliable than otherwise would have been the case.

Although the attempts to improve the seed fertility of the tetraploids in *L. amplexicaule* must be considered very tentative, they did show that real improvements can be made in the seed setting of this species. We shall later have occasion to return to this matter.

B. CYTOLOGY.

Despite numerous fixations, only *a*14 permitted observations of meiosis in tetraploid *L. amplexicaule*. The satisfactory slides obtained were few indeed, and analysis was possible only of I M. Then Benda's modification of Flemming's fixative was employed.

I M was studied in three buds, but the fixation was in no case good enough to permit definite classification of anything but univalents. Bivalents could often be made out too, and occasionally trivalents, for example those V-shaped, and quadrivalents could be made out. However, because as a rule trivalents cannot positively be distinguished from quadrivalents, nor bivalents from trivalents, the classification will in practice be confined to determination for each pollen mother-cell of the number of univalents and of chromosome configurations. According to DARLINGTON's (1937) definition, a configuration is »an association of chromosomes at meiosis, segregating independently of other associations at anaphase», and therefore the term will hereinafter be used collectively to designate bivalents, trivalents, quadrivalents and any valents of higher order, *i. e.* in a sense providing a contradistinction to univalent.

Table 2 lists the found distributions of univalents and configurations and shows how they were distributed on the cells in the buds examined.

The chromosome numbers were not determined in the individual plants. Hence, absolute accuracy should not be expected for valence frequencies calculated from the number of univalents and configurations found per cell. Nevertheless, on the working assumption that all the buds had 36 chromosomes, valence frequencies were worked out for

TABLE 2. *Chromosome pairing at I M in tetraploid L. amplexicaule.*

I + Confs	Bud number 1	2	3
2 + 15		1	
4 + 13	1		
0 + 15		1	
2 + 13			1
0 + 14			1
1 + 13	2	1	1
2 + 12	1		
3 + 11	3		
0 + 13	1	3	2
1 + 12	2	2	2
2 + 11	1		
3 + 10	1		
0 + 12	1	2	1
1 + 11	1	3	1
2 + 10	2	2	
0 + 11	1	3	3
1 + 10	2		
Totals of cells	19	18	12

every bud. Those numerical valence combinations were computed that would arise in the alternative events that (a) every univalent was matched by a trivalent ('strong' pairing) or (b) with an odd number of univalents, only the odd univalent was matched by a trivalent ('weak' pairing). Furthermore, for the sake of comparison, separate valence frequencies were worked out for cells with only a single univalent or none at all — for such cells the theoretical valence frequencies cannot be wrong unless the chromosome number is not 36 or, if 36, it is 'compensated' (36+1 — 1 or the like).

These computations are exemplified in the tabulation below where 13 configurations are associated with 1, 2, 3 and 4 univalents, respectively.

Valences found I + Configs.	Computed frequencies in 'strong' pairing I	II	III	IV	Computed frequencies in 'weak' pairing I	II	III	IV
0+13	0	8	0	5	0	8	0	5
1+13	1	8	1	4	1	8	1	4
2+13	2	8	2	3	2	9	0	4
3+13	3	8	3	2	3	9	1	3
4+13	4	8	4	1	4	10	0	3

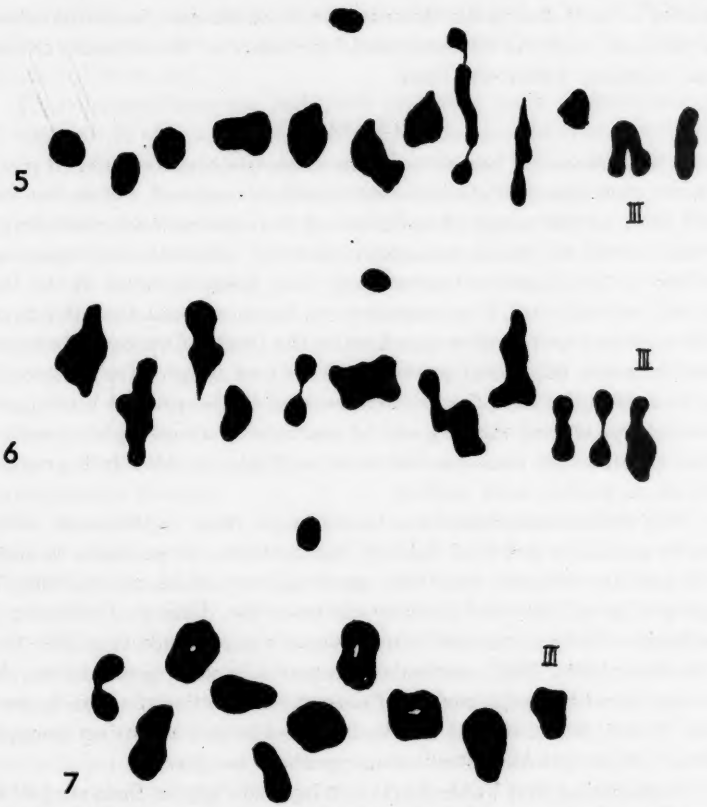
etc.

TABLE 3. *Estimated chromosome associations per cell at I M in tetraploid L. amplexicaule (cf. the text).*

Bud No.	Means of chromosome associations IV	III	II	I	Number of cells	Number of III:s + IV:s per cell
	a) *Maximal number of trivalents (»strong pairing»):*					
1	5,00	1,63	4,74	1.63	19	6,63
2	5,33	0,67	6,00	0,67	18	6,00
3	5,33	0,50	6,33	0,50	12	5,83
	b) *Minimal number of trivalents (»weak pairing»):*					
1	5,53	0,58	5,26	1,63	19	6,11
2	5,50	0,33	6,17	0,67	18	5,83
3	5,42	0,33	6,42	0,50	12	5,75
	c) *Cells having 0 or 1 univalent:*					
1	5,60	0,70	5,40	0,70	10	6,30
2	5,53	0,40	6,13	0,40	15	5,93
3	5,55	0,36	6,18	0,36	11	5,91

The results of the computations are given in Table 3. It reveals that buds 2 and 3 differed negligibly in multivalent frequency (*i. e.* III+IV) whichever way the trivalent frequency was estimated, whereas the difference was greater for bud 1. Buds 1 and 2 in group c had a multivalent frequency falling about halfway between those in groups a and b. This was expected: group c comprised cells selected especially for having valence associations that could have only one interpretation for each cell type with the given chromosome number.

The univalent frequency per cell in bud 1 was about 3 times that in buds 2 and 3, suggesting that its $2n$ might be 35. Cytological observations, however, indicated that bud 1 could not have $2n=35$. Three unusually distinct cells from bud 1 are shown in Figs. 5 to 7, the chromosomes being separately drawn with their mutual levels retained. Fig. 5 shows 12 configs.+1 I and Fig. 6 11 configs.+1 I. If the chromosome number were 35, these ratios of univalents to configurations would not make room for a trivalent. Clearly, however, both cells had a trivalent, as a glance at the illustrations will show. Nor would $2n=35$ admit the presence of a trivalent in Fig. 7 where there are 10 configs.+1 I. Yet the configuration at the extreme right is probably a trivalent. For the present the fact that bud 1 had higher frequencies of univalents as well as of multivalents than buds 2 and 3 must be considered rather an extreme case.



Figs. 5—7. I M groups (drawn separately) in tetraploid *L. amplexicaule*. Every group includes a more or less easily distinguishable trivalent designated III. (See the text.) — ×4500.

Thus we find in *a*14 that each cell had approximately 5,8—6,6 multivalents, 4,7—6,4 bivalents and 0,5—1,6 univalents. The number of quadrivalents could not exceed 9. A more comprehensive investigation would certainly have expanded the range of variation; but, clearly, the proportion of multivalents was rather high for an autotetraploid. This might be the outcome of a fairly high chiasma frequency in conformity with DARLINGTON's (1937) contention that in autotetraploids a high chiasma frequency is positively correlated with a high multivalent frequency.

From the irregular chromosome pairing, one would expect the

gametes to have dissimilar chromosome complements. Such was actually the case, as could be demonstrated by studies of the somatic chromosome numbers within the lines.

C. ANEUPLOIDY.

a. *Chromosomal variation.* — In order to obtain an overall picture of how often aberrant chromosome numbers occurred within the tetraploid lines, of the range of variation of the chromosome numbers and of the extent to which aneuploid numbers affected the vigour and fertility of the plants, a special study was made in three of the lines, *viz.* *a*15, *a*16 and *a*17. For reasons given in the introduction, it was also of interest to ascertain how much wider the limits of aneuploid variation could be made. In general autotriploids are best adapted for such studies, but as autotriploids of the species involved in the present investigation cannot be produced, the progeny of repetitively selected plants with extreme chromosome numbers had to be used instead. Also in this material various properties were studied.

The chromosome numbers found in all these experiments will be seen in sections a and b of Table 4. The mothers of the plants in section a all had $2n=36$ and, relatively speaking, very good seed setting. The high quality of the seed fertility obviated the danger of selecting individuals with 'compensated' chromosome complements (*e.g.* 36+1—1) as mother plants. Thus, section a is intended to show spontaneous chromosome number variations uninfluenced by selection. Section b, on the other hand, demonstrates the variation obtained by using aneuploid mother plants (whose chromosome numbers are given).

Considering first Table 4 a, it will be seen that the lines studied had chromosome numbers ranging from 35 to 39. The modal value was 36, next in order of frequency came 37; 35 and 38 were about as common, and 39 chromosomes were found in but 2 of 188 individuals. In all lines the distribution was markedly skew, with hypertetraploid numbers predominating over hypotetraploid. However, from the percentage sizes of the chromosome number classes, from the overall percentages of aneuploidy and from the mean chromosome numbers, it also appears that mainly *a*17 and in a measure *a*15 had a higher frequency of aneuploids than *a*16, and that these individuals were so distributed as to make more skew the chromosome number distribution in the two former lines than in *a*16. χ^2 for the heterogeneity of the distributions in *a*16 and *a*17 is 6,64 ($0{,}1 > P > 0{,}05$); χ^2 for the association between *a*17 and *a*16 with respect to aneuploidy frequency is 4,52 ($0{,}05 > P > 0{,}02$); χ^2 for the

corresponding association between *a*15 and *a*16 is $3{,}_{17}$ ($0{,}_1 > P > 0{,}_{05}$). Clearly, therefore, the aneuploidy frequency in *a*17 and *a*15 tended to be higher than in *a*16.

These comparisons are justifiable and were made *a priori*, because *a*16 was rich in chasmogamous flowers, whereas cleistogamous flowers were overwhelmingly preponderant in *a*17 and relatively far more numerous in *a*15 than in *a*16. The results of the aforementioned analyses suggest that chromosomally unbalanced gametes, created by the high frequency of multivalents at meiosis, were capable of fertilization more often in *a*17 with its wealth of cleistogamous flowers than in *a*16 where almost half the flowers were chasmogamous. Cleistogamous flowers possess anthers with fewer pollen grains and have shorter styles than chasmogamous flowers; and, being cleistoantheric, the former should also provide conditions more favourable for pollen grain development. The seed of *a*16/44—1 came from plants with profuse chasmogamous flowering, while that of *a*15/45—1 came from 44—2, when *a*15 bore few chasmogamous flowers.

In the light of these experiences one might object that those attempts at widening the chromosome number variation which are entered in Table 4 b should have been done with a cleistogamous line rather than with *a*16. Actually, however, aneuploid plants are, as we shall see below, characterized by a chasmogamous flowering that commences later and is less profuse than in euploid plants, both traits being more pronounced the more the chromosome number departs from the norm. Consequently, the aneuploid mother plants for the most part bore an overwhelming majority of cleistogamous flowers. Moreover, the choice of *a*16 provided an opportunity to study also the pollen fertility of the more extreme aneuploids.

The data in Table 4 b emphasize the skew distribution of the chromosome numbers found in offspring of euploid plants (Table 4 a). It appears, thus, that while the lower limit of chromosomal variation by repeated selection from the offspring of hypotetraploid plants could be shifted from $2n=35$ to $2n=34$, the same procedure moved the upper limit from $2n=39$ to $2n=42$. In either case the expanded range of variation is tantamount to a doubling of the distance designating the spontaneous variation on both sides of the modal value, $2n=36$. The difference in variation on the hypotetraploid and hypertetraploid sides is also reflected in the percentage of aneuploids among progeny of plants with different chromosome numbers. The percentage of aneuploid progeny was about 50 for plants with 34 chromosomes and about

TABLE 4. *Chromosome number variation and class frequencies in tetraploid L. amplexicaule.*

a. *Chromosome numbers in offspring from euploid, well-fertile plants (cf. the text).*

Lines and generations	Number of mother plants	Somatic chromosome numbers 35	36	37	38	39	n	% aneu-ploids	$\bar{x}$
*a*15—45—1	2	4 (8,8 %)	29 (58,0 %)	12 (24,0 %)	4 (8,0 %)	1 (2,0 %)	50	42,0	36,38
*a*16—44—1	5	3 (6,3 %)	36 (75,0 %)	7 (14,6 %)	2 (4,2 %)		48	25,0	36,17
*a*17—44—1	3	1	13	11	4	1	30	56,7	
—44—2	1		10	5	2		17	41,2	
—45—1	4	2	28	11	2		43	34,9	
*a*17, *total*	8	3 (3,3 %)	51 (56,7 %)	27 (30,0 %)	8 (8,8 %)	1 (1,1 %)	90	43,3	36,48

b. *Chromosome numbers in offspring from aneuploid plants (1 mother plant for each progeny).*

Lines and generations	Origin of the mother plant	Number of chroms. of mother plant	Somatic chromosome numbers 34	35	36	37	38	39	40	41	42	n	% aneuploids
*a*16—47													
progeny a	*a*16—44—1	35		4	8	1						13	
» b	»	35	1	4	15	1	1					22	
» c	»	35		1	10	5						16	
			1	9	33	7	1					51	35,3
» d	»	38			9	9	10	3	1			32	
» e	»	38		1	3	5	5	—	3			17	
				1	12	14	15	3	4			49	75,5
*a*16—48													

				1	12	14	15	3	4				49	75,5	
*a*16—48															
progeny a		*a*16a—47	35	3	4	9	2	2	1	1	1		23		
»	b	»	35	1	4	16	3	1					25		
				4	8	25	5	3	1	1	1		48	47,9	
»	c	*a*16c—47	36[1]		2	5	5	15	7	4	1		39	87,2	
»	e	*a*16d—47	39					1	1				2		
»	f	»	39				2	—	1				3		
							2	1	2				5	100	
»	g	*a*16d—47	40			1							1		
»	h	*a*16e—47	40					2					2		
»	i	»	40				1	2	4	5	2	2	16		
»	k	»	40						2	2	—	1	5		
						1	1	4	6	7	2	3	24	95,8	
*a*16—49															
progeny a		*a*16a—48	34			9	2	4	2				17		
»	b	»	34		3	7	1	2					13		
»	c	»	34	1	1	3	—	1					6		
»	d	*a*16b—48	34		6	14	5	1					26		
				1	10	33	8	8	2				62	46,8	
»	e	*a*16a—48	41			12	3	1					16		
»	f	*a*16c—48	41			3	2	9	1				15		
						15	5	10	1				31	51,6	
»	g	*a*16i—48	42				2	11	3	—	—	1	17		
»	h	»	42			2	—	4	1	4	3	1	15		
»	i	*a*16k—48	42							1	—	3	4		
						2	2	15	4	5	3	5	36	94,4	

[1] The plant was highly sterile, and probably an aneuploid of »compensated» type (*cf.* the text).

75 for plants with 38 chromosomes. All, or almost all, the offspring of plants with higher chromosome numbers were aneuploids, the sole exception being the progeny of plants having 41 chromosomes, and particularly the offspring of one such plant. It turned out, however, that these plants were themselves so aberrant in many features peculiar to the other highly hypertetraploid plants (of which more below) that one might doubt the correctness of the chromosome number as determined for at least one of these plants. Progeny c of *p*30/49, derived from a 41-chromosome plant, however, showed no small resemblance to progeny e of *a*16/49. Hence considerable variation can occur, and circumspection should be observed.

One should not expect consistently to find the same chromosome number distribution throughout the offspring of several plants with the same aberrant chromosome number. This will be dependent on which chromosomes or chromosome constellations are responsible for the overrepresentation or underrepresentation as the case may be (see below). More or less characteristic examples of this occurred in the progeny of plants having 34 and 42 chromosomes. For example, in progeny a of *a*16/49 the mean chromosome number was $36{,}_{94} \pm 0{,}_{27}$, whereas progeny d of the same line and generation had $36{,}_{04} \pm 0{,}_{15}$ for its mean chromosome number. The *t*-value for the difference between these means is $2{,}_{85}$ ($0{,}_{01} > P > 0{,}_{001}$). Similarly, progenies g and h of *a*16/49 were found to have mean chromosome numbers of $38{,}_{29} \pm 0{,}_{27}$ and $39{,}_{20} \pm 0{,}_{47}$, respectively, but the *t*-value for their difference is only $1{,}_{68}$ ($P \simeq 0{,}_{1}$).

A drastic example of what the constitution of the mother plant can mean for the composition of the offspring occurred in *a*16/48, progeny c. Of these plants, 87 per cent were in fact aneuploids, as against 25 per cent for *a*16 in Table 4 a. Although the mother plant of progeny c, 1948, had $2n=36$, a number of its properties aroused the suspicion that it might represent an extreme case of 'compensated' chromosome complement. Evidently the composition of the progeny justifies the suspicion. With respect to those mother plants having 34 chromosomes mentioned in the preceding paragraph things are not so straightforward, however. There the aberrations of the mother plants might have been due solely to absence of different chromosomes, or constitutions of 'compensated' type could have been a contributory factor. In the example where the mother plants had 42 chromosomes excess of different chromosomes and dissimilar 'compensations' were perhaps jointly responsible.

In the face of comparisons and examples such as those given above, and for any evaluation whatever of the aneuploidy data collected in

Table 4 b, one should constantly keep in mind that these data in all probability represent merely a tiny fraction of the aneuploid chromosomal combinations possible in the species. The table reveals that there was a very narrow basis for selection of the aneuploids. Such a material cannot be regarded as particularly representative of the chromosomal variation possible within the species; at best it can provide a rough idea thereof. But that was actually all the experiments were designed to do.

To believe that the available data truly reflect the maximal intraspecific variability of the chromosome numbers would most likely lead one astray. Probably different groups of genes influence gametic and zygotic viability unequally in hypertetraploidy and hypotetraploidy, whether they alone represent the aberrant portion of the chromosome complement or occur in association with other aberrations of the chromosome number. Considering the narrow basis for the experiments done, it seems more likely than not that the chromosome constellations most favourable for extreme aneuploidy numbers failed to materialize, or that such gametes at best had occasion for unilateral function. As a guess, therefore, one may conclude that the chromosomal variation in the tetraploid *L. amplexicaule* might have a somewhat greater range. Such a view is in fact borne out by the ease whereby the extreme chromosome numbers $2n=34$ and $2n=42$ were produced after but two generations of aneuploid selection and with a very narrow basis for selection in the present experiments.

b. *Effects of chromosomal variation.* — The aforementioned qualifications are valid also for studies of the properties of aneuploids — the properties warrant no conclusions that would be upset by any minor changes of the results that might have arisen had the material studied been more comprehensive. In offspring from euploid plants a study was made of the influence of the chromosome number on seed setting, pollen fertility, plant height and florescence time; and in offspring from aneuploid plants the following additional factors were observed: degree of vegetative development and number of leaf indentations. The observations made are assembled in Tables 5 and 6. Before these tables are discussed, it seems appropriate to repeat the statement made in the introduction to the effect that these experiments sometimes had to yield precedence to more important projects, and that some characters consequently were followed up for a generation or two while others came in for more thorough observation. Here another warning should be made: owing to the impermanence of the material, some plants had occasionally begun to wither or otherwise become un-

TABLE 5. *Correlations between chromosome number*

Lines and generations	$2n = 34$		$2n = 35$		$2n = 36$		$2n = 37$	
	$\bar{x} \pm m$	n	$\bar{x} \pm m$	n	$\bar{x} \pm m$	n	$\bar{x} \pm m$	n
a. *Offspring from euploid, well-fertile plants (correspond to those of Table 4, a).*								
1. Seed setting.								
*a*15—45—1			16,0 ± 2,35	4	46,2 ± 1,32	29	33,8 ± 2,26	12
*a*16—44—1			30,2 ± 5,13	3	42,2 ± 1,04	34	34,8 ± 3,09	6
*a*17—44—1			41	1	60,6 ± 2,94	13	56,6 ± 2,53	11
*a*17—44—2					54,6 ± 3,30	15	54,5 ± 4,99	8
*a*17—45—1			35,0 ± 2,00	2	63,3 ± 1,44	28	61,5 ± 1,67	11
2. Pollen fertility.								
*a*15—45—1			87,7 ± 1,33	4	89,7 ± 0,56	28	87,0 ± 1,20	11
*a*16—44—1			89,1 ± 0,35	2	93,1 ± 0,46	32	91,2 ± 0,70	7
*a*17—45—1			94	1	91,6 ± 0,82	14	92,8 ± 1,02	4
3. Height of stem.								
*a*15—45—1			40,0 ± 2,68	4	41,2 ± 0,88	26	39,8 ± 1,36	11
*a*17—44—1			48	1	50,2 ± 1,26	13	45,8 ± 1,61	9
*a*17—44—2					40,7 ± 2,42	11	44,2 ± 3,68	5
*a*17—45—1			40,5 ± 0,50	2	36,4 ± 0,81	26	37,4 ± 1,22	10
b. *Offspring from aneuploid plants (correspond to those of Table 4, b).*								
1. Seed setting.								
*a*16—47	38	1	31,1 ± 4.05	10	44,8 ± 2,17	45	34,2 ± 3,06	21
*a*16—48	24,1 ± 3,26	4	28,0 ± 4,57	10	41,8 ± 2,17	31	39,3 ± 3,48	13
*a*16—49	14,8	1	38,2 ± 3,17	10	47,8 ± 2,08	50	44,4 ± 3,59	15
2. Pollen fertility.								
*a*16—47	88	1	86,9 ± 0,72	9	89,7 ± 0,67	43	89,3 ± 0,90	18
*a*16—48	79,7 ± 2,30	4	80,6 ± 2,01	10	83,7 ± 1,50	31	82,2 ± 1,81	12
3. Height of stem.								
*a*16—47	37	1	35,3 ± 1,98	9	33,9 ± 1,09	43	31,5 ± 1,98	20
*a*16—48	29,7 ± 2,33	3	30,3 ± 1,68	9	31,7 ± 0,98	28	28,9 ± 1,76	12
*a*16—49	42	1	44,7 ± 2,63	10	45,7 ± 0.92	50	48,3 ± 2.70	15
4. Vegetative development.								
*a*16—48	2,2 ± 0,75	4	2,4 ± 0,31	10	1,9 ± 0,17	31	2,2 ± 0,25	13
*a*16—49	2	1	1,6 ± 0,22	10	1,7 ± 0,11	50	1,7 ± 0,18	15
5. Indentations per leaf.								
*a*16—48	8,58 ± 0,220	3	8,50 ± 0,433	6	9,14 ± 0,164	23	9,27 ± 0,216	12
*a*16—49	8,75	1	8,05 ± 0,443	5	9,72 ± 0,234	23	9,72 ± 0,319	9

and different characteristics in tetraploid L. amplexicaule.

$2n = 38$		$2n = 39$		$2n = 40$		$2n = 41$		$2n = 42$	
$\bar{x} \pm m$	n	$\bar{x} \pm m$	n	$\bar{x} \pm m$	n	$\bar{x} \pm m$	n	$\bar{x} \pm m$	n
a. *Offspring from euploid, well-fertile plants (correspond to those of Table 4, a).*									
1. Seed setting.									
31,5 ± 5,95	4	21	1						
28,0 ± 1,25	2								
53,1 ± 4,35	3	24	1						
39,6 ± 6,02	5								
40,0 ± 4,00	2								
2. Pollen fertility.									
82,0 ± 4.37	4	90	1						
89,7 ± 4,20	2								
3. Height of stem.									
38,7 ± 2,03	3	39	1						
38,0 ± 9,08	4	39	1						
29,2 ± 8.35	5								
27,0 ± 12,00	2								
b. *Offspring from aneuploid plants (correspond to those of Table 4, b).*									
1. Seed setting.									
21,1 ± 2,63	16	2,3 ± 0,93	3	1,3 ± 0,49	4				
29,8 ± 2,41	23	18,4 ± 3,69	16	21,2 ± 6,15	12	40,3 ± 6,12	4	9,2 ± 7,20	3
39,2 ± 2,76	33	35,6 ± 5,99	7	21,4 ± 5,75	5	2,1 ± 6,20	3	21,0 ± 3,42	5
2. Pollen fertility.									
86,5 ± 1,49	14	83,6 ± 2,89	3	79,7 ± 3,51	4				
74,4 ± 1,95	23	75,2 ± 3,39	12	75,0 ± 3,36	11	82,4 ± 4,96	4	74,0 ± 3,30	3
3. Height of stem.									
30,4 ± 1,85	16	32,2 ± 5,07	3	30,1 ± 1,23	4				
31,6 ± 1,10	22	31,0 ± 1,97	11	27,5 ± 2,27	12	31,3 ± 3,20	4	28,3 ± 6,91	3
44,8 ± 1,74	33	42,3 ± 4,13	7	49,6 ± 3,87	5	52,3 ± 11,57	3	39,4 ± 5,32	5
4. Vegetative development.									
1,7 ± 0,19	23	2,3 ± 0,27	16	2,7 ± 0,36	12	2,0 ± 0,00	4	2,3 ± 0,67	3
1,7 ± 0,16	33	2,0 ± 0,22	7	1,6 ± 0,25	5	2,3 ± 0,67	3	2,6 ± 0,40	5
5. Indentations per leaf.									
8,99 ± 0,331	19	8,95 ± 0,210	11	8,68 ± 0,214	11	9,44 ± 0,277	4	7,92 ± 0,441	3
8,91 ± 0,207	19	9,10 ± 0,504	5	8,25 ± 0,102	4	6,75 ± 0,750	2	8,33 ± 0,333	3

suitable for observation of one character or another by the time such observations could be made. That is why there were so few individuals in some groups.

Among the characters studied, seed setting has proved most susceptible to variations in chromosome number. Through all lines and generations seed setting was best in the $2n=36$ class. The superiority over 35-chromosome plants was significant for practically all generations intralinearly; and the same was true for 37-chromosome plants in a few instances, but these displayed a seed setting that was less reduced compared with the euploid group than in 35-chromosome plants. The seed fertility in several generations of 37-chromosome plants was about the same as but not better than in the corresponding 36-chromosome plants in all of the eight comparisons — even if it was a close shave once. The chance of this happening is only 1 in 2^8 $(=256)$. On the whole, therefore, the acquisition of an extra chromosome reduced the seed setting significantly.

Table 5 shows, furthermore, that two extra chromosomes $(2n=38)$ reduced the seed fertility about as much as the loss of one chromosome $(2n=35)$. With still higher chromosome numbers the seed setting apparently underwent a further progressive impairment. However, the several classes comprised insufficient individuals and the material itself was inadequate for a more detailed analysis, so we shall have to be satisfied with the general traits mentioned in the above. Their implications will be considered later in another context. The 1948 lot of 41-chromosome plants had a surprisingly high seed setting and, as mentioned previously in connection with their progeny, there are good grounds for believing that the estimated chromosome number was erroneous for an occasional plant.

In all the generations studied the pollen fertility was highest for euploid plants, except in *a*17/45—1 which comprised few plants and where $2n=37$ showed a slightly higher pollen fertility than $2n=36$. Apart from this the aneuploid plants were characterized, as in the case of seed setting, by progressively reduced pollen fertility the more the chromosome number deviated from euploidy. This gradual impairment was with few exceptions very evident. The available results leave no doubt that a significant negative correlation existed between increasingly aberrant chromosome numbers and decreasing pollen fertility.

With respect to pollen fertility one can perceive the same tendency which was so clearcut in the case of seed setting: that in comparison with the euploid group plants with 35 chromosomes if anything were

Figs. 8—9. Tetraploid *L. amplexicaule*. Mature aneuploid plants of varying vigour (see the text).

inferior to those with 37. Thus, in pollen quality too, the 35-chromosome group was more like that with 38 than that with 37 chromosomes, so that the loss of one chromosome was as influential as the addition of two extra chromosomes.

Even if, as we have seen, both the seed setting and the pollen fertility were progressively reduced as the degree of aneuploidy inclined, these two expressions of fertility were markedly dissimilar in one respect. For each degree of aneuploidy the reduction in terms of per cent of the seed setting far exceeded that of the pollen fertility. Considering now the four plant generations embracing lines *a*15 and *a*16 for which seed setting as well as pollen fertility were recorded, we find that the 35-chromosome group averaged 61 per cent of the euploid plants' seed setting and 97 per cent of their pollen fertility. Similarly, those individuals having 37 chromosomes had, respectively, 82 and 98 per cent of the euploid group's seed setting and pollen fertility, while the corresponding figures for plants with 38 chromosomes were 63 and 94 per cent. The probable cause of these differences will be dealt with below.

To express the vigour the height of the plant's stem was the best available yardstick susceptible of measurement. True, weighing of the plants would have been preferable, but this proved technically impossible. In a few generations attempts were made to replace weighing of the plants with assessment of vegetative development in accordance with a four-unit scale where the strongest plants were assigned a '1' and the weakest a '4'. A less rough scale proved difficult to handle in practice. Figs. 8 and 9 illustrate a plant selected at random from each of these developmental classes, and they should provide an idea of the differences involved.

The height of the stem was not by far so strongly correlated with

the chromosome number as the fertility. Among the generations shown in Table 5 a, such a correlation emerges in two of the lines, *a*15/45—1 and *a*17/44—1, but not in the two others. In the various generations of *a*16 in Table 5 b, no direct correlations can be discerned between chromosome number and stem height. Nevertheless, the height of the stem was in a degree affected by aneuploid chromosome numbers, for the mean height of all the aneuploids was in most cases lower than that of the euploid plants of the same generation. This comes out in the scheme reproduced below.

	All euploid plants	n	All aneuploid plants	n
*a*15/45—1	41,2 ± 0,88	26	39,6 ± 0,96	19
*a*17/44—1	50,2 ± 1,26	13	43,4 ± 2,57	15
*a*17/44—2	40,7 ± 2,43	11	36,7 ± 4,98	10
*a*17/45—1	36,4 ± 0,81	26	36,4 ± 1,88	14
*a*16/47	33,9 ± 1,09	43	31,8 ± 1,03	53
*a*16/48	31,7 ± 0,98	28	30,1 ± 0,70	76
*a*16/49	45,7 ± 0,92	50	45,4 ± 1,17	79

Although the euploid plants were taller than the aneuploid in 6 of the 7 comparisons shown above, the differences were so small that a slightly significant superiority was attained in a single case only (*a*17/44—1; $t=2{,}38$). However, if the mean of the differences between the means for the euploid and the aneuploid plants is calculated, the quotient between it and its standard error will be 2,63 ($0{,}05 > P > 0{,}02$). This shows that the euploids with some degree of probability were taller than the aneuploids (*cf.* FISHER, 1938, p. 127).

A similar situation obtained with regard to vegetative development. One can discern in Table 5 b a slight correlation between the latter property and increasing chromosomal unbalance; the association becomes clearer if the aneuploids are classified by chromosome number, as follows:

	$2n=34$—35	36	37—38	39—42
*a*16/48	2,36 ± 0,289	1,90 ± 0,169	1,89 ± 0,153	2,40 ± 0,180
*a*16/49	1,64 ± 0,203	1,70 ± 0,108	1,73 ± 0,122	2,10 ± 0,176

It will be seen that in both generations only the strongly hypertetraploid plant groups tended to be less well developed vegetatively than the euploid plants. Even if neither generation included a significant difference, the fact that this tendency recurred suggests that the hypertetraploids at least were characterized by inferior vegetative development, just as the aneuploids evidently were slightly less tall than the euploids.

Because many plants whose characters suggested they were an-

euploids had leaves with a subnormal number of marginal teeth, these — well developed and of varying size in *L. amplexicaule* — were counted on the two lowermost pairs of leaves on the inflorescence of the stem. A glance at the table will show that the number of indentations per leaf was associated with the degree of aneuploidy through two generations. The 41-chromosome plants of the 1948 generation constituted a glaring exception but, as we have already seen, they were atypical in many other respects and therefore suspect. A diminished number of indentations per leaf has also been observed in aneuploids of *L. intermedium* (BERNSTRÖM, 1953 b) as well as in a synthetic allopolyploid between *L. hybridum* and *L. amplexicaule* (BERNSTRÖM, unpublished), so the phenomenon would seem to be no rarity in this genus.

Lastly the chromosome number's relationship to florescence was studied. Some of the findings will be found in Table 6. The various groups of *a*17/44—2 and *a*17/45—1 were too small for inclusion in the table and will instead be mentioned below. The following procedure was used. At inspections on alternate days in 1944 and 1945 and every third day in 1947 and 1948, flowering plants were recorded. The florescence time for each group was then expressed as the average number of days elapsed from the first till the last plant had burst into flower. Occasional plants were excluded from the analysis because slow germination had caused them to be potted a day or two after their group fellows. In *L. amplexicaule* florescence begins with the appearance of cleistogamous flowers; only a week or two later do the first chasmogamous flowers of the summer generation break out.

The cleistogamous florescence proved on the whole correlated with the degree of aneuploidy, euploid plants usually bearing the earliest flowers. The mean florescence time for euploids in *a*17/45—1 was 1,9 days, *versus* 2,9 days when 2*n* was 37. In *a*17/44—2 plants with 36 and 37 chromosomes had approximately the same mean florescence times (4,4 and 4,3 days, respectively), whereas those with 38 chromosomes on the average flowered after 5,0 days. As appears from Table 6, 37-chromosome plants in *a*15/45—1 were if anything as early as the corresponding euploids. By and large, however, the more normal the chromosome number the earlier was evidently the florescence.

The chasmogamous florescence showed a similar tendency. In Table 6, all the 3 generations show a clear correlation between rising aneuploidy and delayed chasmogamous florescence; and among the plants flowering chasmogamously in *a*17/45—1 florescence was on the average a day earlier for individuals with 36 chromosomes than for

TABLE 6. *Correlation between chromosome number and florescence time in tetraploid L. amplexicaule (cf. the text).*

2n	Lines and generations													
	*a*15—45—1				*a*16—47					*a*16—48				
	Cleistog. flows.	n	Chasmog. flows.	n	Cleistog. flows.	n	Chasmog. flows.	n	n[1]	Cleistog. flows.	n	Chasmog. flows.	n	n[1]
34					4	1	20	1	0	6,3 ± 2,25	4	16,0 ± 1,22	4	0
35	8,5 ± 0,87	4	17,5 ± 1,50	4	5,1 ± 1,41	9	16,5	6	3	4,7 ± 0,67	9	15,0 ± 0,71	9	0
36	6,9 ± 0,46	29	17,3 ± 0,77	29	2,5 ± 0,38	41	15,0	41	0	4,5 ± 0,32	30	14,0 ± 0,30	30	0
37	6,5 ± 0,50	12	18,3 ± 0,75	12	5,2 ± 0,82	17	15,9	11	6	5,4 ± 0,55	13	16,2 ± 0,79	13	0
38	8,0 ± 1,00	3	19,0 ± 1,73	3	6,0 ± 0,96	15	16,3	12	3	5,0 ± 0,46	22	14,9 ± 0,61	22	0
39	7	1	28	1	7,0 ± 0,00	3	20	1	2	7,5 ± 0,62	12	16,6	10	2
40					9,7 ± 1,33	3	20,0	2	1	7,8 ± 0,91	12	17,0	9	3
41										5,5 ± 0,87	4	14,5 ± 1,50	4	0
42										6,0 ± 1,00	3	18,0 ± 2,00	3	0

[1] Plants still without chasmogamous flowers at the end of the observation period.

those with 37. Not all the plants bore chasmogamous flowers. This was the case of *a*16/47 and in a measure also of *a*16/48, for both of which generations Table 6 gives the number of plants that had produced no chasmogamous flowers at the end of the observation period. The length of the observation period was 21 to 22 days. The mean chasmogamous florescence times for the incompletely studied chromosome number classes in these generations would therefore if anything be on the low side and, hence, the correlation stronger than it ostensibly is.

Unfortunately it proved impractical to make as detailed notes as would have been desirable of the quantity of chasmogamous flowers on each plant. Most likely it would then also have appeared that particularly aneuploid plants produced much fewer chasmogamous flowers than the euploid plants. Numerous observations and notes suggest this.

At this point it is of interest to keep in mind the fact that what we have seen in the foregoing applies to the general features of aneuploid plants in the species studied. Among living plants, however, aneuploid specimens can often be distinguished by morphological aberrations other than those mentioned. Thus, in addition to weaker growth which often also becomes more stunted or in other ways habitually deviates from normal, aneuploids frequently have leaves of different shape and structure, and not seldom their calyces and flowers acquire an abnormal appearance. No systematic investigations into the relationship between chromosome number and habitual characteristics were carried out, and to do so would have been rather pointless. Yet the observations made were sufficient to show that the loss or acquisition of a single chromosome usually failed to reflect itself in any clear external signs. Those 35-chromosome plants which could be singled out as being chromosomally aberrant were merely characterized by a somewhat subnormal vegetative development and occasionally by a remarkably small number of marginal leaf indentations, whereas the majority of plants with 37 chromosomes lacked all morphological signs thereof. Accordingly, one should expect external abnormalities only in association with fairly considerable chromosomal aberrations. And with respect to an autotetraploid species it is hardly surprising that an extra chromosome lost or gained affects the external characteristics so vaguely.

2. L. PURPUREUM.

A. FERTILITY.

The tetraploids were inferior to the diploids in pollen fertility, the former being about 91 to 98 per cent as pollen fertile as the latter (*cf.*

TABLE 7. *Pollen fertility in lines of tetraploid*

Generations	44–1			44–2			45–1		
Lines	$\bar{x}$	n	in % of the diploid	$\bar{x}$	n	in % of the diploid	$\bar{x}$	n	in % of the diploid
p6	91,9	20	93	92,4 ± 0,34	21	94			
p7	90,2	90	92	92,4 ± 0,38	29	94	92,0 ± 0,62	40	95
p30	91,6	27	92	90,4	23	91	93,0 ± 0,50	46	95
p42				92,0	7	94	94,5 ± 0,58	27	97
p43									
p59									
p60									
p61									
p62									
Hybrids	$\bar{x}$	n	in % of the mean of the parents	$\bar{x}$	n	in % of the mean of the parents	$\bar{x}$	n	in % of the mean of the parents
p6 × *p7*				F_1 : 94,2 ± 0,88	6	102			
p7 × *p30*							F_2 : 96,0 ± 0,21	28	103
p7 × *p42*							F_2 : 94,6 ± 0,53	26	101
p30 × *p43*									
p42 × *p43*									
p59 × *p62*									
p60 × *p61*									

Table 7). While probably no inheritable differences obtained between lines, the fertility occasionally displayed a between generations difference. In explaining this one cannot reject the possibility that the various observers of the pollen samples might have applied slightly dissimilar criteria to the classification of and distinction between pollen grains of different kinds. Consequently, it would be unwise to invest such differences with any significance. Attempts to establish the true ratio of viable to inviable pollen grains by germination of the pollen *in vitro* will be mentioned in due course.

The pollen fertility of interlinear hybrids was also studied, and the results are given in Table 7. The fact that these hybrids were intended mainly for use in hybridization experiments is the reason why, as the table reveals, they were produced several times over. Since lack of time occasionally made it necessary to skimp these experiments, which were

L. purpureum, and in their hybrids.

45—2			47			49		
$\bar{x}$	n	in % of the diploid	$\bar{x}$	n	in % of the diploid	$\bar{x}$	n	in % of the diploid
			95,3	16	—	93,0 ± 0,92	14	95
93,7 ± 0,75	31	—	94,6 [1]	32	—	94,5 ± 0,76	14	—
94,3 ± 1,10	10	96	94,4 ± 1,35	7	97	91,4 ± 1,14	15	94
91,5 ± 1,05	20	93	94,9 ± 0,71	16	96	96,7 ± 0,42	14	98
						95,4 ± 0,39	17	—
						98,3 ± 0,39	14	—
						97,3 ± 0,71	13	98
						97,7 ± 0,41	13	—
$\bar{x}$	n	in % of the mean of the parents	$\bar{x}$	n	in % of the mean of the parents	$\bar{x}$	n	in % of the mean of the parents
F_1 : 94,1	5	—				F_1 : 97,9 ± 0,56	8	104
F_2 : 95,5	18	—				F_1 : 96,9 ± 0,32	6	105
F_1 : 93,4 ± 1,29	13	101				F_1 : 95,8 ± 0,68	13	100
F_1 : 97,1 ± 0,31	13	105	F_1 : 94,7 ± 2,59	9	100	F_1 : 98,0 ± 0,48	12	104
						F_1 : 96,4 ± 0,58	12	100
						F_1 : 97,5 ± 0,72	5	100

[1] *p*30—47 is based on equal parts of C_3 and C_5 plants ($\bar{x} = 93{,}3$ and 95,9, respectively).

carried out alongside the main investigation, and since some of the F_1 generations merely comprised one or two plants, the data included in the table are not as complete as one would have wished. Nevertheless, it clearly appears that the pollen fertility in no case failed to match the averaged means for the parent lines. On the contrary at least 4 of the 7 hybrid combinations examined exceeded and the rest equalled the parent lines in pollen fertility.

In view of the fact that diploid hybrids corresponding to the tetraploid combinations studied here have a pollen fertility that is quite unimpaired and intermediate between those of the parent lines (BERNSTRÖM, 1953 b), one would expect the tetraploid hybrids to behave in much the same way. In fact, however, the found tendency suggests that hybrids commonly will have better pollen quality than their parents. The data are unfortunately inadequate for a conclusive statistical

TABLE 8. *Seed setting in lines*

Generations	44—1				44—2				45—1			
Lines	$\bar{x}$	in % of the diploid	n	C-generation	$\bar{x}$	in % of the diploid	n	C-generation	$\bar{x}$	in % of the diploid	n	C-generation
p6	38,1 ± 2,02	40	21	C4	37,9 ± 2,02	41	24	C5	42,3 ± 1,36	45	23	C6
p7	37,4 ± 0,87	40	103	C6	39,4 ± 1,31	41	32	C7	41,3 ± 1,04	43	44	C8
p30	22,3 ± 1,81	24	23	C1	31,5 ± 2,00	33	25	C2	28,6 ± 1,36	32	49	C3
p42					26,3 ± 3,55	32	9	C1	27,7 ± 1,49	31	29	C2
p43									37,4 ± 2,14	40	22	C2
p59												
p60												
p61												
p62												

analysis of found and expected values. Yet it is an irrefutable fact that 2 of the hybrid combinations had a pollen fertility significantly superior to the best parent line's, thus:

F_1 *p7* × *p30/49*	*versus*	*p30*, $t=3{,}62$	$(0{,}01 > P > 0{,}001)$
F_2 *p7* × *p30/45—1*	»	*p30*, $t=5{,}57$	$(P < 0{,}001)$
F_1 *p7* × *p42/49*	»	*p7*, $t=4{,}02$	$(P < 0{,}001)$
F_1 *p42* × *p43/45—2*	»	*p42*, $t=2{,}28$	$(0{,}05 > P > 0{,}02)$
F_1 *p42* × *p43/49*	»	*p43*, $t=2{,}03$	$(0{,}1 > P > 0{,}05)$

Considering the circumstance that two of the three F_1 generations of *p42* × *p43* studied showed a superiority with some significance over the best of the respective parent lines, it is very likely that in this hybrid, too, the pollen fertility had undergone a real improvement. As the pure lines had pollen fertilities which in themselves were rather high, it is remarkable that among the 7 hybrid combinations studied as many as 3 evidently had a better pollen fertility than any of the parent lines.

Data on the seed setting in tetraploid lines of *L. purpureum* are given in Table 8. It will be seen that, compared with the diploids, the tetraploids had a far lower seed setting; at best it was only 50 per cent as high. This means that tetraploid *L. purpureum* was not a little inferior to *L. amplexicaule*, where the pure lines could attain 65 per cent of the diploids' seed setting. The dissimilarity might well be due simply

of tetraploid L. purpureum.

45—2				46				47				49			
$\bar{x}$	in % of the diploid	n	C-generation	$\bar{x}$	in % of the diploid	n	C-generation	$\bar{x}$	in % of the diploid	n	C-generation	$\bar{x}$	in % of the diploid	n	C-generation
$39{,}6 \pm 1{,}57$	—	19	C9	$34{,}7 \pm 1{,}60$	—	24	C9	$24{,}0 \pm 2{,}37$	--	16	C10	$45{,}9 \pm 3{,}47$	49	12	C12
$41{,}1 \pm 1{,}41$	48	29	C4					$23{,}0 \pm 3{,}61$	—	16	C3	$41{,}9 \pm 2{,}61$	—	14	C4
								$29{,}0 \pm 3{,}42$	—	16	C5				
$31{,}9 \pm 2{,}98$	35	14	C3	$33{,}8 \pm 1{,}63$	42	15	C4	$24{,}2 \pm 5{,}35$	33	7	C5	$34{,}5 \pm 3{,}59$	—	15	C7
$40{,}4 \pm 2{,}64$	45	20	C3	$40{,}7 \pm 2{,}04$	—	24	C3	$38{,}4 \pm 2{,}70$	42	14	C4	$50{,}2 \pm 2{,}59$	—	14	C6
												$45{,}6 \pm 1{,}76$	—	17	C3
												$30{,}8 \pm 1{,}96$	—	14	C3
												$50{,}5 \pm 1{,}49$	—	13	C3
												$31{,}5 \pm 1{,}70$	—	13	C3

to the fact that *L. purpureum* lacks cleistogamous flowers, whereas *L. amplexicaule* has them in plenty. Moreover, just as in *L. amplexicaule*, differences were found within lines between generations as well as between lines within generations in the seed setting of *L. purpureum*.

Intralinear intergenerational seed-setting differences should in most cases probably be ascribed to seasonal variation. The most typical examples of this were the years 1947 and 1949. The summer was very sunny and hot in 1947 and, in addition, the plants were then growing in an unusually dry and warm greenhouse, in fact the same as was used in 1946. On the other hand, the house used in 1949 was much better ventilated and more spacious, and during the flowering period the weather was normal. Thus the environmental conditions for the plants in 1947 differed considerably from those in 1949. It is not unreasonable to suppose that at least part of the seed-setting difference between these two years was brought about by these environmental dissimilarities. In 1944 and 1945 the plants were in adequately ventilated frames with open sides and glass tops.

In evaluating the seed setting of tetraploid *L. purpureum* the possible influence of nectarivorous Hymenoptera must be taken into due account. We have already seen that insects far oftener visit the flowers of tetraploid *L. purpureum* than of the diploids or of other species. We have

seen, too, that about 5 per cent of the flowers are incapable of self-fertilization. In 1944, 1945 and 1949 insects could and did freely visit the flowers, whereas in 1946 and 1947 the glass-house was screened off with fine-meshed netting so that the insects could gain access to the plants in exceptional circumstances only. The low seed setting in almost all the 1947 lines, as well as the reduced seed fertility of *p*7 in 1946, might well have resulted partly from the exclusion of insects and not be wholly dependent on growing conditions. Of course the preceding statement should not be construed to mean that a high incidence of cross-fertilization is normal: also in tetraploids vicinism is, as pointed out previously, merely a sporadic phenomenon. Yet the insects (predominantly little bumble-bees) just by droning around from one flower to another may easily bring about self-fertilization where it has not happened already.

A markedly atypical reaction to altered environmental conditions in 1946 and 1947 was presented by *p*43. From generation to generation this line had a very even seed fertility; as in the other lines it increased in 1949 only. Perhaps the seed setting in this line escaped impairment in 1946 and 1947 because it had a more reliable self-fertilizing mechanism than the other lines. Conversely *p*42 had a seed setting which not only underwent a reduction in 1947 but, generation by generation, showed a more or less significant inferiority to that of *p*43. Here we evidently have to do with an inherited seed-setting difference between different pure lines. However, the discrepancy cannot be attributed to unlike responses to chromosome number doubling in the seed setting of different lines, for a tendency towards lower seed-setting capacity than in other lines was already discernible in *p*42's diploid ancestral line *p*26 (BERNSTRÖM, 1950 a).

The great and significant seed-setting difference between the first and second generations of *p*30 in 1945 was most likely due to spontaneous cross-fertilization between lines, probably with *p*6 or *p*7 and originating in the first generation in 1944. For it was noticed in 1945 that the line no longer exhibited all those characteristics which formerly had been typical of it and instead displayed some of the features seen in lines *p*6 or *p*7; in 1945—2 these changes were so pronounced that some of the plants could have been classified with the latter lines or with *p*30 with equal justification. In 1944—2, owing to the lack of clearcut morphological differences between *p*6 and *p*30, it was overlooked that several plants could be hybrids. Consequently, in selecting the most fertile plants — as we shall see below, interlinear hybrids of *L. purpureum* very often have a remarkably high fertility — these hy-

TABLE 9. *Seed setting in interlinear hybrids of tetraploid L. purpureum.*

Cross combination	$\bar{x} \pm m$	n	No. of filial generation	Year and plant generation	Seed setting in % of	
					the mean of the parents	the best parent line
*p*6 × *p*7	44,4 ± 5,42	7	F_1	44—2	115	113
»	41,9 ± 1,81	22	F_2	45—1	100	99
*p*7 × *p*30	40,5 ± 3,58	6	F_1	45—2	100	99
»	42,5 ± 3,93	8	F_1	49	97	93
»	44,6 ± 1,24	28	F_2	45—1	128***	108*
»	37,7 ± 1,81	17	F_3	45—2	93	92
*p*7 × *p*42	36,7 ± 4,70	5	F_1	44—2	112	93
»	56,0 ± 6,10	6	F_1	49	139*	122
»	45,7 ± 2,37	26	F_2	45—1	132***	111(*)
»	46,6 ± 2,20	19	F_3	45—2	130***	118*
*p*30 × *p*42	49,0 ± 1,44	4	F_1	45—2	134***	119***
»	40,5 ± 1,67	20	F_2	45—2	111(*)	99
*p*30 × *p*43	38,0 ± 3,13	15	F_1	45—2	95	95
»	50,6 ± 3,07	13	F_1	49	110	101
*p*42 × *p*43	51,5 ± 1,62	15	F_1	45—2	142***	127**
»	37,1 ± 5,36	9	F_1	47	119	97
»	62,5 ± 2,52	12	F_1	49	148***	125**
*p*59 × *p*62	56,5 ± 2,83	12	F_1	49	147***	124**
*p*60 × *p*61	60,7 ± 2,01	5	F_1	49	148***	120***

Levels of *t*-significance for differences between the hybrid mean and the averaged parent means or, respectively, the best parent mean (for methods, consult BERNSTRÖM, 1952, p. 179) denoted thus: (*) $P < 0{,}1$. — * $P < 0{,}05$. — ** $P < 0{,}01$. — *** $P < 0{,}001$.

brids would most likely be selected. Moreover, as each generation comprised offspring of several plants, most of them numbering only a few individuals, it is not possible in retrospect to ascertain which plants up to 1945—2 actually were hybrids and which were pure. C_3 of *p*30/47 was the progeny of a pure line, but C_5 was composed of hybrid offspring. *p*30/49 was derived from C_3 of *p*30/47.

As it happened, the seed setting was studied more thoroughly than the pollen fertility in interlinear hybrids of tetraploid *L. purpureum*. The findings made will be seen in Table 9. It reveals that in the 8 hybrid combinations studied the seed setting was superior, usually clearly so, to the average for the parent lines in 6 and equivalent therewith in the remaining 2. In only 3 generations of altogether 2 hybrid combinations, the mean seed setting expressed as a percentage of the averaged means

for the parent lines was less than 100. It should be noted, however, that the two lower relative values were attached to hybrids involving *p*30 and studied in 1945—2, when part of the cultivated *p*30 specimens were heterozygous from outcrossing and probably had a higher seed fertility than the pure *p*30 would have had. Accordingly, if these 2 instances are disregarded as probably being erroneous, there remains but a single F_1 generation derived from *p*7 and *p*30 and having a seed setting of 97 per cent. Since all the other relative numbers were equal to or exceeded 100, the seed setting of the hybrids was evidently characterized by the same general tendency to rise above the average level for the pure lines as was displayed by the pollen fertility.

With respect to 4 cross combinations the seed fertility was already good enough in the F_1 hybrids to constitute a fairly or highly significant improvement upon that of the better parent line. The 1947 F_1 generation of *p*42×*p*43 admittedly showed an opposite tendency; but, as mentioned, in 1947 the seed setting of *L. purpureum* was abnormal and therefore these results should not be given the same weight as those for the other two years when the same cross combination was studied. Apparently little if any fertility improvement was to be found in the F_1 generations of *p*7×*p*30 (evaluation complicated by *p*30/45—2) and *p*7×*p*42, but already in the F_2 generations the seed setting fairly significantly exceeded even that of the better parent line. While the same applied to F_2 of *p*7×*p*42, F_2 of *p*7×*p*30 and also F_2 of *p*30×*p*42 were distorted by *p*30/45—2.

Thus, to recapitulate, we find that the seed setting of tetraploid *L. purpureum* actually can be improved by interlinear hybridization and also that similar improvement probably can be attained by means of selection when the seed fertility of an F_1 generation shows no sign of superiority. Unfortunately the intervention of more important duties made it impossible to carry these studies any further. It would have been interesting to ascertain, for example, how the seed setting would have benefitted from combined hybridization in the present material. A more penetrating analysis would also have shed more light than do the present findings on the relationship, if any, between increased pollen fertility and improved seed setting. The existence of such a relationship not only seems a reasonable assumption, it is also indicated by the results. Were it not for the extremely high pollen fertility values for some lines in 1949 there might have been a regular tendency, and a more comprehensive material would have informed us whether the high pollen fertility or the apparent correlation was due to random variation.

TABLE 10. *Chromosome pairing at diakinesis in tetraploid L. purpureum.*

I+	0	1	0	1	0	1	0	3	2	1	0	4	2	1	Total
+Conf:s :	10	10	11	11	12	12	13	11	12	13	14	11	13	14	of cells
Number of cells :	1	1	4	3	3	4	2	1	1	4	1	1	1	3	30

B. CYTOLOGY.

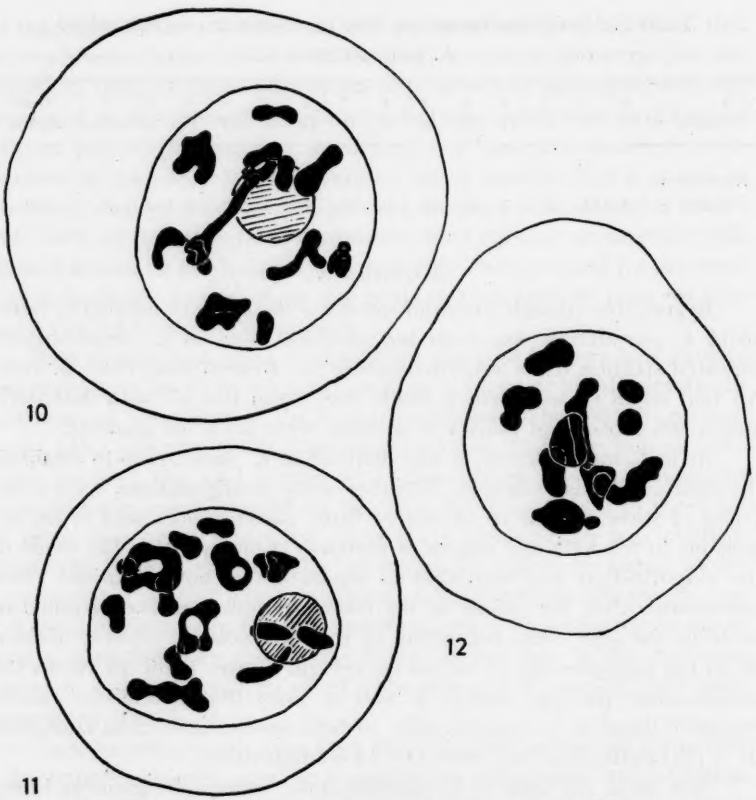
Regrettably enough, material suited for the study of meiosis in tetraploid *L. purpureum* was even less plentiful than in *L. amplexicaule*, repeated fixation trials notwithstanding (*cf.* BERNSTRÖM, 1952, p. 166). All that could be used was a single slide from line *p7* with diakinetic stages; all subsequent phases of division were unfit for analysis.

As in *L. amplexicaule*, it was difficult in *L. purpureum* to establish the configurational valences. Whether some configurations were composed of three or four or of two or three chromosomes was often impossible to tell with any degree of certainty. Consequently, the scope of the classification was restricted to separation of configurations from univalents. After the nature of the configurations had been studied in some of the best cells, estimation of the chromosome number yielded 36 as the probable but by no means certain figure. Table 10 shows the chromosome pairings found. It will se been that conditions closely resemble those in *L. amplexicaule*: in both species most cells contained 0—1 univalents and, as a rule, 11—14 configurations.

Just as in the case of *L. amplexicaule*, valence frequencies based on the probable chromosome number 36 were calculated for *L. pur-*

TABLE 11. *Estimated chromosome associations per cell at I M in tetraploid L. purpureum* (*cf. the text*).

Means of chromosome associations IV	III	II	I	Number of cells	Number of III:s + + IV:s per cell
a) *Maximal number of trivalents (»strong pairing»):*					
5,07	0,87	6,13	0,87	30	5,94
b) *Minimal number of trivalents (»weak pairing»):*					
5,23	0,53	6,30	0,87	30	5,76
c) *Cells having 0 or 1 univalent:*					
5,31	0,58	6,23	0,58	26	5,89



Figs. 10—12. PMC's of tetraploid *L. purpureum* at diakinesis. — Fig. 10, 11 confs. +1 I; Fig. 11, 14 confs.+1 I; Fig. 12, 10 confs.+1 I. The cell in Fig. 12 was at a slightly later stage than the others. — ×4500.

pureum. The results will be found in Table 11. The values obtained conform closely with those in *L. amplexicaule*. Thus, judging by the single successful slide, there were also in *L. purpureum* about 6 multivalents per cell. The average in *L. amplexicaule* ranged from 5,8 to 6,6; and, though most uncertain, the corresponding figure in *L. purpureum* was clearly rather high. Figs. 10—12 depict three of the cells inspected (*cf.* captions to Figs.).

C. ANEUPLOIDY.

a. *Chromosomal variation.* — The chromosomal variation and its limits were analysed in *L. purpureum*, using the same procedure as in

L. amplexicaule, the results obtained being collected in Table 12 a and b. As in the corresponding table for *L. amplexicaule* (Table 4), section a is intended to show the chromosomal variation in offspring from euploid plants, while section b does the same thing for offspring from aneuploid plants.

Lines *p*7 and *p*30 were used for these experiments. The various *p*30 generations entered in Table 12 a were, as mentioned, not pure but more or less heterozygotic from outcrossing with other lines since preceding generations. While the majority of those plant offspring of *p*30/45—1 which were studied cytologically presumably were pure, *p*30/45—2 was probably composed mostly of heterozygotic plants. This should be duly considered in evaluating results obtained from *p*30.

As most of the aneuploids in both lines were hypertetraploids, the chromosome number distribution was skew — which, as we have seen, was also the case in *L. amplexicaule*. Numerically speaking less prominent than in *p*7, the skewness in *p*30 was accentuated through the presence of three plants having 38 chromosomes. Strange to say, such plants were wholly lacking in *p*7. This was probably a random difference between the lines. As will be seen, 38-chromosome plants may occur spontaneously in *p*7.

Compared with the aneuploid distribution found in tetraploid *L. amplexicaule* (Table 4 a), it is remarkable that plants with 39 chromosomes were lacking in *L. purpureum* where, besides, the chromosome number distribution on the whole seems less skew than in *L. amplexicaule*. On the other hand it should be noted that in *a*16 and *p*30 the distributions showed fair agreement. Thus the interspecific difference, if it is a real difference, might equally well be due merely to the absence of cleistogamous flowers in *L. purpureum*. However, these studies were far from comprehensive enough to warrant a point by point comparison between the species; to do so would have required more lines than those available for the present investigation.

Fewer attempts to extend the range of chromosome number variability were made in *L. purpureum* than in *L. amplexicaule*. In most cases *p*30 was used. (Two offspring from hypertetraploid *p*7 plants were produced for an early pilot experiment but never studied thoroughly.) The 38-chromosome plant used as the progenitor for aneuploidy experiments with *p*30 belonged to the C_2 generation and was pure *p*30 (*p*30/44—2); and its offspring retained the line characteristics. These experiments suffer from the weakness of not having started from a wider assortment of aneuploids. Here the same reservations and limit-

TABLE 12. *Chromosome number variation and class frequencies in tetraploid L. purpureum.*

a. *Chromosome numbers in offspring from euploid, well-fertile plants (cf. the text).*

Lines and generations	Number of mother plants	Somatic chromosome numbers 35	36	37	38	n	% aneu-ploids	$\bar{x}$
*p*7—44—1	2	4	24	8		36		
—44—2	4	1	21	10		32		
—45—1	5	3	29	11		43		
*p*7, *total*		8 (7,2 %)	74 (66,7 %)	29 (26,1 %)		111	33,3	36,19
*p*30—45—1	3	4	27	5	2	38		
—45—2	3	5	18	7	1	31		
*p*30, *total*		9 (13,0 %)	45 (65,2 %)	12 (17,4 %)	3 (4,3 %)	69	34,8	36,13

ations must consequently apply as were made in discussing the aneuploidy experiments with *L. amplexicaule*.

The results obtained are given in Table 12 b. It will be realized that *p*30's chromosome number variability ranged quite as widely as *a*16's (Table 4 b). Actually its range was slightly wider considering that a plant with 43 chromosomes was found. For reasons similar to those given with respect to *L. amplexicaule*, one may surmise that the range found does not represent the ultimate limits of variation in *L. purpureum*. Thus, one may expect in progenies of different aneuploid plants with the same chromosome number to find — and, as will be seen, in both species one actually finds — discrepant chromosome number distributions, depending on which particular chromosomes were supernumerary or lacking. This, combined with the fact that the aneuploids obtained — particularly the more extreme ones — most probably represented but a small proportion of all the combinations possible within the various chromosome numbers and a variability fully as wide as that in *L. amplexicaule* nevertheless was found in just four progenies derived from a single 38-chromosome plant, all goes to show that the observed variability was not a maximum.

The chromosome number variation in *L. purpureum* greatly resembled that in *L. amplexicaule*. Thus a shift towards hypertetraploidy was much commoner than one towards hypotetraploidy and, except for the 43-chromosome plant mentioned previously, the chromosome numbers varied within the same limits in both species. The two species

TABLE 12. *Chromosome number variation and class frequencies in tetraploid L. purpureum.*

b. *Chromosome numbers in offspring from aneuploid plants (1 mother plant for each progeny).*

Lines and generations	Origin of the mother plant	Number of chroms. of mother plant	Somatic chromosome numbers										n	% aneu-ploids
			34	35	36	37	38	39	40	41	42	43		
*p*7—43—2	C_4-gen.	38		1	4	9	12	1					27	85,2
*p*7—44—1	*p*7—43—2	39			1	1	7	2					11	90,9
*p*30—47														
progeny e	C_2-gen.	38		2	7	11	10	2	1				33	78,8
*p*30—48														
progeny b	*p*30e—47	35	1	1	10	1	1	4	—	2			20	
» c	»	35	1	2	18	2	1	1					25	
			2	3	28	3	2	5	—	2			45	37,8
» e	»	39			1	1	16	3	2	1	1	1	26	96,2
» f	»	40			2	1	8	1	2	1			15	86,7
*p*30—49														
progeny c	*p*30b—48	41		4	10	2	3	2					21	
» d	*p*30f—48	41					8	7	3	1	2		21	
				4	10	2	11	9	3	1	2		42	76,2
» e	*p*30e—48	42				2	3	4	4	5			18	100
» f	»	43		1	4	3	5	—	1				14	71,4

seemed also to agree well with respect to the percentages of aneuploids in progenies of plants with equally aberrant chromosome numbers. In *L. purpureum* the offspring from plants with very high chromosome numbers might have included rather more 36-chromosome plants than in *L. amplexicaule*, but this could well be due exclusively to the constitution of the individual mother plants and need not be characteristic of the species as a whole. We have already seen in *L. amplexicaule* some examples of how the constitution of the mother plants influences the chromosome number distribution in their offspring; in *L. purpureum* it is interesting to note that the two 41-chromosome mother plants had progenies whose chromosome number distributions differed very significantly ($t=6{,}8$), and also that progeny f of *p*30/49 — even though the mother plant's chromosome number was 43 — averaged 37,14 chromosomes, while the corresponding figure in the same year was 39,39 in progeny e whose mother plant had but 42 chromosomes. Further, the aforementioned progeny f's mean was significantly lower than that for progeny d in the same year, *viz.* 39,14 ($t=4{,}5$). Common to both species was the tendency that the higher the plant's chromosome number the higher would be the mean chromosome number in the progeny, which makes it all the more remarkable that in 1949 progeny f on an average had a lower chromosome number than progenies d and e in the same year. Clearly, then, the chromosome number distribution in progenies of aneuploid plants is dependent not only on the chromosome number of the mother plant but also on its chromosomal constitution. Had more, and more populous, plant progenies been available, the same thing would probably have appeared also in offspring from plants having less hypertetraploid chromosome numbers than had the mother plants in our examples.

b. *Effects of chromosomal variation.* — In dealing with this subject, it is well to recall the reservations and remarks made in the corresponding section on *L. amplexicaule*, for they apply to *L. purpureum* with equal justification. In the latter species the same characters were analysed as in *L. amplexicaule*, the number of leaf teeth excepted. The leaves of *L. purpureum* are regularly crenate-serrate and so unsuited for such a study.

Before we come to the relationships between chromosome number and the degree of various characters, another point should be stressed which perhaps was passed over too lightly in connection with *L. amplexicaule*, *viz.* the composition of the group with plants having 36 chromosomes. Among descendants of euploid plants, the large majority

of those with 36 chromosomes assumedly have four complete chromosome sets. On the other hand it is likely that a number of 36-chromosome individuals derived from aneuploid plants are aneuploids themselves and have 'compensated' chromosome numbers, chiefly of the pattern $36+1-1$. In order to find the frequency of such plants, if it can be found, it would be necessary to study meiosis of each plant separately, which would be technically impossible in the species concerned. Nor can said frequency be deduced from the chromosome number distribution in the offspring: the end result would be influenced by too many unknowns, *e. g.* the range of chromosome number variability in the viable gametes on the male and female sides, respectively, the chromosome number class frequencies on these sides, the growth rate of the pollen tubes from pollen in different chromosome number classes, incidence of zygotic elimination, and probably other factors as well.

The chromosome number distributions found nevertheless warrant the conclusion that gametes having at least 17—22 chromosomes were capable of functioning. Considering the susceptibility of pollen grains to deficiencies and duplications, it seems rather unlikely that pollen more aberrant than $\pm 1(-2)$ chromosomes can function; on the other hand, it seems reasonable to suppose that pollen no more aberrant than ± 1 chromosome should be able to function, as the double chromosome complement of diploid pollen grains should make them far less susceptible than haploid grains (*cf.* below). The chromosome numbers found would not have arisen unless the ovular chromosome numbers had had a range of variability greater than that mentioned. Hence, the chromosome numbers of the pollen as a whole must also have ranged within wider limits. It follows that 36-chromosome plants commonly will arise after combination of gametes with 17 and 19 chromosomes, respectively, and the more aberrant the chromosome numbers in the mother plants, the commoner will be 'compensated' 36-chromosome plants in the offspring. Consequently, in correlating chromosome numbers with different characters, it would be wrong to expect 36-chromosome plants in offspring from aneuploids to be comparable with those having euploid mother plants, or to expect the former to be as superior to the adjacent chromosome number classes as the latter. Admittedly the groups with 35 and 37 chromosomes, particularly the latter, probably also included plants with 'compensated' chromosome complements but in proportionately lower numbers. The low hypotetraploidy frequency in progenies of 35-chromosome plants indicates that, in these plants, sharper genetic and/or zygotic elimination will suppress the compensating group.

TABLE 13. *Correlations between chromosome number and*

Lines and generations	$2n = 34$		$2n = 35$		$2n = 36$		$2n = 37$	
	$\bar{x} \pm m_{\bar{x}}$	n	$\bar{x} \pm m$	n	$\bar{x} \pm m$	n	$\bar{x} \pm m$	n
a. *Offspring from euploid, well-fertile plants (correspond to those of Table 12, a).*								
1. Seed setting.								
p7—44—1			26,4 ± 3,35	4	40,1 ± 1,22	23	33,0 ± 3,55	7
p7—44—2			33	1	42,0 ± 1,44	16	32,4 ± 2,70	6
p7—45—1			34,0 ± 4,73	3	44,1 ± 0,87	29	36,6 ± 2,30	11
p30—45—1			21,5 ± 0,65	4	27,6 ± 1,23	27	21,6 ± 4,13	5
p30—45—2			35,7 ± 4,11	4	43,7 ± 1,74	18	37,2 ± 2,27	6
2. Pollen fertility.								
p7—44—1[1]			89,6 ± 1,17	4	91,7 ± 0,68	11	89,0 ± 0,70	6
p7—44—2			92	1	93,0 ± 0,47	15	91,3 ± 0,98	5
p7—45—1			94,9 ± 1,60	3	94,0 ± 0,37	27	89,4 ± 2,09	9
p30—45—1			90,7 ± 1,01	4	94,2 ± 0,55	25	94,0 ± 0,59	5
p30—45—2			90,7 ± 2,07	5	94,7 ± 0,99	18	93,0 ± 1,29	7
3. Height of stem.								
p7—44—1			31,0 ± 3,03	4	37,0 ± 1,04	24	31,3 ± 2,72	8
p7—44—2			29	1	35,5 ± 2,30	15	24,4 ± 2,84	7
p7—45—1			27,7 ± 1,76	3	32,4 ± 0,72	29	28,6 ± 1,66	11
p30—45—1			24,8 ± 2,72	4	24,6 ± 0,91	27	24,4 ± 0,40	5
p30—45—2			32,0 ± 3,63	5	34,7 ± 2,14	14	33,0 ± 3,30	7
b. *Offspring from aneuploid plants (correspond to those of Table 12, b).*								
1. Seed setting.								
p30—47			12,0 ± 3,70	2	30,3 ± 3,70	7	27,0 ± 3,62	11
p30—48	32,3 ± 10,90	2	31,7 ± 7,38	3	34,8 ± 1,45	31	29,1 ± 3,52	5
2. Pollen fertility.								
p30—47			92,1 ± 0,90	2	96,2 ± 0,78	7	94,4 ± 0,46	11
p30—48	94,0 ± 1,40	2	92,8 ± 2,44	3	94,7 ± 0,61	31	89,7 ± 4,56	5
p30—49			92,8 ± 1,29	5	90,7 ± 1,42	14	93,6 ± 2,36	7
3. Height of stem.								
p30—47			—	—	48,6 ± 3,17	5	40,9 ± 1,96	9
p30—48	39,5 ± 0,50	2	41,7 ± 1,33	3	39,1 ± 0,88	31	32,4 ± 1,50	5
p30—49			37,0 ± 5,41	5	38,8 ± 3,16	14	39,7 ± 3,19	7
4. Vegetative development.								
p30—48	1,5 ± 0,50	2	1,3 ± 0,33	3	1,5 ± 0,09	31	2,2 ± 0,20	5
p30—49			1,6 ± 0,40	5	1,5 ± 0,20	14	1,6 ± 0,30	7

[1] Some plants were never studied.

different characteristics in tetraploid L. purpureum.

2n = 38		2n = 39		2n = 40		2n = 41		2n = 42	
$\bar{x} \pm m$	n	$\bar{x} \pm m$	n	$\bar{x} \pm m$	n	$\bar{x} \pm m$	n	$\bar{x} \pm m$	n
a. ***Offspring from euploid, well-fertile plants (correspond to those of Table 12, a).***									
1. Seed setting.									
15,0 ± 9,00	2								
40	1								
2. Pollen fertility.									
91,3 ± 4,65	2								
96	1								
3. Height of stem.									
20,0 ± 1,00	1								
41	2								
b. ***Offspring from aneuploid plants (correspond to those of Table 12, b).***									
1. Seed setting.									
22,7 ± 2,30	10	19,1 ± 16,58	2	8	1				
28,7 ± 1,79	26	22,3 ± 2,46	9	22,2 ± 3,83	4	18,5 ± 2,74	4	7	1
								(2n = 43; 20	1)
2. Pollen fertility.									
93,8 ± 0,61	10	92,7 ± 0,95	2	89	1				
89,8 ± 1,05	26	88,0 ± 1,99	9	79,9 ± 10,07	4	83,7 ± 2,56	4	83	1
								(2n = 43; 88	1)
86,3 ± 2,53	19	86,0 ± 2,47	13	88,7 ± 1,82	8	83,1 ± 2,92	6	78,8 ± 2,25	2
3. Height of stem.									
42,0 ± 2,68	7	32	1	20	1				
33,2 ± 1,35	26	34,8 ± 2,50	9	25,3 ± 0,87	3	34,0 ± 3,58	4	20	1
								(2n = 43; 33	1)
36,3 ± 1,55	19	33,4 ± 2,23	13	30,6 ± 2,08	8	23,8 ± 2,55	6	28,0 ± 3,00	2
4. Vegetative development.									
2,3 ± 0,12	26	2,3 ± 0,24	9	3,3 ± 0,33	3	1,8 ± 0,48	4	4	1
								(2n = 43; 3	1)
2,0 ± 0,17	19	2,2 ± 0,20	13	2,6 ± 0,18	8	3,0 ± 0,37	6	2,5 ± 0,50	2

Tetraploid *L. purpureum* exhibited characteristic responses to aberrant chromosome numbers resembling those in tetraploid *L. amplexicaule*. As shown in Table 13, seed setting was also in *L. purpureum* the property responding most sharply to chromosome number increases or decreases. Thus, in both a and b, plants with 36 chromosomes consistently had the best seed setting, and the difference between the groups with 36 and 37 chromosomes in degree of seed setting was throughout even more marked in *L. purpureum* than in *L. amplexicaule*. With increasingly aberrant chromosome numbers the seed fertility showed a steady decline in both generations of aneuploid offspring within *p*30. Evidently, therefore, in *L. purpureum* as well as in *L. amplexicaule*, there existed a marked negative correlation between rising degrees of aneuploidy and declining seed-setting ability.

Exactly the same pattern was observed for the pollen fertility, just as was the case in *L. amplexicaule*. The pollen fertility values in tetraploid *L. purpureum* were in general slightly higher than in tetraploid *L. amplexicaule*, and the aneuploid groups showed a similar tendency, but the degree of pollen fertility impairment associated with increasingly aberrant chromosome numbers was about the same in the two species. *p*30/49 was the only generation where these two properties were imperfectly correlated. In particular the pollen fertility of the $2n=36$ plants was unexpectedly low, but then the entire generation was derived from strongly hypertetraploid plants. The mother plants of all other generations of aneuploid offspring were in both species partly hypotetraploid, partly hypertetraploid; and, as mentioned, one may expect in 36-chromosome offspring of hypotetraploid plants a much larger proportion of euploid plants than in offspring derived from strongly hypertetraploid plants. It is much to be regretted that the seed fertility could not be studied in *p*30/49, for it ought to have provided the best confirmation of this explanation of the atypical behaviour of the pollen fertility.

The tendency for both the seed fertility and the pollen fertility of plants having 35 chromosomes to be more subnormal than those of plants with 37 chromosomes was less manifest in *L. purpureum* than in *L. amplexicaule*. None the less, however, in *L. purpureum*, too, most groups with 35 chromosomes were inferior to those with 37. This is an interesting point because in other species one finds that the vigour in particular is reduced more by the loss of a chromosome than by the acquisition of a supernumerary chromosome, whereas a similar action on the fertility is seen more rarely. On the other hand, it is hard to find

any such difference with regard to height of stem or vigour in either of the species studied here (*cf.* Tables 5 and 13).

The height of the stem was found to be clearly associated with the chromosome number in 4 of the 5 generations derived from euploid mother plants, plants with 36 chromosomes being taller than those with 35 or 37. Occasionally the differences between groups with 36 and groups with 37 chromosomes were slightly significant. Plants with 35 chromosomes were too few in number for statistical treatment. Plants in the different groups had approximately the same height in *p*30/45—1 only, although the mean for all aneuploids was merely 23,7 as against 24,6 for the euploid group.

A correlation between chromosome number and plant height could also be discerned in offspring from aneuploid mother plants, at least in so far as the hypertetraploid groups almost without exception were less tall than 36-chromosome plants, and the plant height on the whole tended to diminish with increasing chromosome numbers. The hypotetraploid chromosomal groups exhibited no such tendency, but they comprised too few individuals to warrant any conclusions in either direction. When the plants with 36 chromosomes were compared within generations with all aneuploids, the former proved distinctly taller. Thus:

	All euploid plants	n	All aneuploid plants	n
*p*30/47	48,6 ± 3,17	5	40,0 ± 1,90	18
*p*30/48	39,1 ± 0,88	31	33,5 ± 0 94	54
*p*30/49	38,8 ± 3,16	14	33,9 ± 1,09	60

Accordingly, in all of the eight generations, the euploids proved taller than the aneuploids, and so the tendency of the euploids to be taller seems to have a fairly solid basis. On the whole, plants with a normal chromosome number in *L. purpureum* were more clearly superior to the aneuploids in plant height than was the case in *L. amplexicaule* (*cf.* Table 5 and p. 204).

The situation was similar with respect to degree of vegetative development. Table 13 b reveals the presence of a marked correlation between rising chromosome number aberration and diminished vigour, even if the tendency is somewhat upset in *p*30/48 through the presence of two flourishing plants having 41 chromosomes. After the data have been broken down in the same way as for *L. amplexicaule* (p. 204) the correlation emerges even more distinctly:

	34—35	36	37—38	39—43
*p*30/48	1,40 ± 0,25	1,55 ± 0,09	2,32 ± 0,11	2,50 ± 0,22
*p*30/49	1,60 ± 0,40	1,50 ± 0,20	1,88 ± 0,15	2,52 ± 0,14

The correlation is here upset only by the hypotetraploid group in *p*30/48, but the group comprised merely 5 plants. Actually the differences between the euploids and the various hypertetraploid groups were fully significant in both generations except for plants with 37 and 38 chromosomes in 1949 which with a very low degree of probability ($0{,}2 > P > 0{,}1$) were less vigorous than plants having a normal number of chromosomes. Generally speaking, however, in *L. purpureum* the hypertetraploid plants evidently were less vigorous than those with 36 chromosomes, the more so the more aberrant the chromosome number.

The time of florescence in 1944—2, 1945—1 and 1948 of both *p*7 and *p*30 was calculated in the same way as in the case of *L. amplexicaule*. Strangely enough, however, no clearcut and consistent difference between euploid and aneuploid plants emerged as regards florescence. The only sign of a relationship between chromosome number and florescence time might be the fact that in 1948 plants with 40 chromosomes and over began to flower a day or two later than the others. In *L. amplexicaule*, on the other hand, increasingly aberrant chromosome numbers were clearly associated with later florescence. It will be realized, therefore, that the two species behaved altogether differently in this respect.

Probably oftener in *L. purpureum* than in *L. amplexicaule* the aneuploids were characterized by deviating habitual features which, in addition to plant height and vigour, as a rule involved the appearance of leaves or calyces, their shape, indentations, *etc.* Examination of plants, not least those of *p*7, whose chromosome number was later to be determined disclosed that over half the aneuploids could be picked out on the basis of external features alone, but in so doing about 15 per cent of the euploid plants were mistakenly classified as aneuploids. The plants thus selected included none with 35 chromosomes, but several of those with 37 and most of those with 38 chromosomes. A 36-chromosome plant with uncommonly low fertility also had deviating external features; its chromosome complement was probably of 'compensated' type. Obviously, aneuploids are easier to distinguish in *L. purpureum* than in *L. amplexicaule* owing to the very different appearances of the two species, *i. a.* as regards such habitual features as the size of, and the number of serrations on, the leaves.

Minor discrepancies in floral colour and shape were by no means rare in tetraploid *L. purpureum*. Probably, and in many cases certainly, aneuploid chromosome numbers were the underlying cause; but it was not possible to study this variation closely.

IV. DISCUSSION.

1. FERTILITY.

A general finding is that new autotetraploids have lower seed fertility than the diploids. The diminution may vary but it is always present. Its causes are associated with two main factors: (a) the unsuitability, due to doubling of the chromosome number, of the genotype for the organism of the autotetraploids; and (b) the meiotic irregularities engendered by the presence of 4 in place of 2 homologous chromosome sets.

The genotypic unbalance can be briefly explained thus. Products of a long process of selection, the original forms represent delicately poised systems of genotype, chromosome number and organism, as organized and controlled by the genotype. Doubling of the chromosome number must have upset the former state of equilibrium. Without exception the cell volume increases in new autotetraploids; and cells about twice as large necessarily mean anatomical and physiological changes that rhyme ill with the genotype. A point probably having significance in this connection was brought up by SCHWANITZ (1950). He pointed out that the cellular surface-volume ratio in the tetraploids was about 20 per cent lower than in the diploids, which would seem to affect adversely the metabolism and interfere with various vital processes including the fertility. The autotetraploids' genotypic unbalance and its different manifestations have been discussed by various authors, including MÜNTZING (1936) and LEVAN (1945 a), who emphasized that all those disturbances of one kind or another which accompany a doubling of the chromosome number must be detrimental to processes so delicate as the formation of sex cells and the fertilization process.

Disturbances at meiosis, *i. e.* disturbances following chromosome number doubling, are caused by chromosome pairing in trivalents and quadrivalents. The existence of such valents leads to the formation of gametes with discrepant chromosome numbers, and of these at least the more extreme ones will have a lethal or inhibiting effect on their bearers. This reason for partial sterility, which DARLINGTON (1937) has given, may presumably be largely responsible for the sterility of the autotetraploids; but which of the factors mentioned is dominant in different species is usually imperfectly understood. A significant contribution to this matter has been made by HÅKANSSON and ELLERSTRÖM (1950) in a paper dealing with the seed setting of tetraploid rye. It was found that the reduced fertility was due not so much to haplontic

sterility as to the combined effects of absent fertilization and disturbed and interrupted seed development. Why this was so is obscure, but the factors mentioned in the preceding paragraph were probably in part responsible (*cf.* below).

Judging by the present investigation, the reduced fertility of autotetraploids in *Lamium* was due both to chromosomal and to genic abnormalities. In both species the multivalent frequency seemed quite as high, relatively, as in autotetraploids of other genera, and therefore numerous gametes, particularly the male gametes, could be expected to be unfit for fertilization. Even if only the male gametes suffer from aberrant chromosome numbers, the seed fertility must suffer, too, when pollination is substandard. Indeed, in tetraploid *L. amplexicaule*, self-fertilization failed in 30—40 per cent of the chasmogamous flowers, as against 10 per cent in the diploids. In fact, the seed setting in the chasmogamous flowers of the tetraploids tends to improve if self-pollination is ensured (BERNSTRÖM, 1950 b). In tetraploid *L. purpureum* up to 5 per cent only of the flowers were unpollinated compared with 1—2 per cent in the diploids. However, it should be kept in mind that reduced self-fertilization is parallelled by a reduction in the average number of pollen grains that have been transferred to the stigma of self-fertilized flowers. The fact that the tetraploids have pollen grains twice as large as the diploids means, too, that on the whole fewer pollen grains will compose the masses — held together by pollen cement — that are transferred from the anthers to the stigma. Another factor contributing to a less efficacious pollination must be the fact stressed by SCHWANITZ and SCHWANITZ (1950) that the pollen grains formed in the anthers of autotetraploids are only about two-thirds as numerous as those of the diploids. The above-mentioned conditions were very much in evidence when the self-fertilization rate was studied in the tetraploids. Thus, flowers with just a few pollen grains on the stigma were no rarity; and, if a high proportion of the grains were non-functional (see below), then the seed setting must have suffered.

Another thing shown in the paper cited above (BERNSTRÖM, 1950 b) is that about 40 per cent of the reduction of the seed fertility of the tetraploid *a*55's chasmogamous flowers could be attributed to irregularities in that phase of the fertilization process which commences with pollen germination and concludes with completion of the fertilization. This rough estimate was possible owing to the presence of both cleistogamous and chasmogamous flowers. In these circumstances it was assumed that the cleistogamous flowers, which in addition are cleistoantheric, came

very close to reflecting the true seed-setting power of the line. It was further assumed that the gynaecia of both types of flowers were equivalent. The pollination of the chasmogamous flowers was checked. It is interesting to note that the findings of this investigation bear out HÅKANSSON and ELLERSTRÖM's results — in tetraploid rye, too, some of the loss of fertility was due to non-fertilization. Similar results have been obtained by COOPER and BRINK (1945) in tetraploid *Lycopersicon pimpinellifolium* and by EINSET (1944) in tetraploid *Lactuca sativa*.

In *Lamium*, as in a number of other species, doubling of the chromosome number has resulted in a reduction of the pollen fertility far less marked than that characterizing the seed fertility. Considering that the male gametes in several species have proved more susceptible than the female gametes to deficiencies, duplications and the like, one would have expected in autotetraploids to find the opposite of what has been found. The explanation of this apparent anomaly must, however, probably be looked for in the fact discussed above that autotetraploids to a large extent have both haplontic and diplontic sterility. Similarly, some of the pollen grains, though apparently sound, may actually be incapable of functioning. The latter is of course but one phase of the sterility itself. LAMM (1944), for example, found that a triploid rye plant having 83 per cent of apparently good pollen grains (after staining of the same type as was used in *Lamium*) did not function as male parent in crosses with diploids. A diploid twin plant had 96 per cent of good pollen. By means of experiments *in vitro* with a number of species from various genera, SCHWANITZ (1942) demonstrated that pollen from autotetraploids germinated in a considerably lesser extent than pollen from diploids, in spite of the best germinating conditions having been developed for each kind of pollen from every species. In addition the diploid pollen tubes always were shorter than the haploid ones and a large percentage of them burst.

These results fit in remarkably well with the corresponding findings in experiments with *Lamium* pollen. After trying several methods and rejecting them in favour of a modification of KOKHANOVSKAYA's (1939) procedure, the present author found in repeated experiments a germination percentage of 96 for pollen from *p*25 compared with barely 60 per cent for pollen from *p*30. Actually the procedure adopted was so reliable that in duplicate germination experiments with pollen from a special set of 10 plants in *p*30/45—2 the differences between individual plants were very similar in the two experiments. The means for all the plants were 59,8 and 56,5 per cent, respectively, whereas the average germination

percentage per plant ranged from 30 to 76,5. The great interindividual variability was most likely due to the fact that the *p*30 generation used had been rendered strongly heterozygotic by previous outcrossing. Now it turned out that in *Lamium*, too, the diploid pollen tubes were much shorter and less uniformly developed than the haploid, and they very often burst. It should here be added that of pollen grains from the tetraploid species *L. hybridum* and *L. intermedium* about 92 and 80 per cent, respectively, germinated and mostly had longer and better developed pollen tubes than was the case in tetraploid *L. purpureum*.

Although the artificial background of these results lays them open to some reservations, it should be noted that they confirm the aforementioned experiences of frequent fertilization failures in autotetraploids. Thus some of the pollen, despite its apparent good quality, seems in fact to have been wholly or almost wholly incapable of functioning. It is unclear whether this pollen characteristic resulted from aneuploid chromosome numbers — which must evidently have been present in a considerable proportion of the seemingly good pollen grains — or was an expression of the same type of genotypic unbalance as in the adult plants. It is probably safe to guess that both factors were at work jointly (*cf.* p. 232); and, presumably, the poor pollen quality may easily be more marked by gynaecial disturbances due to genotypic unbalance.

The improved seed setting in autotetraploids following interlinear hybridization is a phenomenon which now is known to occur in many genera. Thus, MÜNTZING (1943 a) demonstrated that interlinear crossing of autotetraploids of *Galeopsis pubescens* with selection in the progeny in due course almost doubled the seed fertility. Similar experiences have been reported in barley (MÜNTZING, 1943 b), flax (LEVAN, 1945 a; KUCKUCK and LEVAN, 1951), vetches (NORDENSKIÖLD, 1953) and numerous other plants. The reason why the seed fertility improves following hybridization and selection must, at least partly, be ascribed to gene combinations more compatible with the tetraploid state than the original genotype. The increased fertility would then admit of the same explanation as the greater yields from autotetraploids of various cultivated plants after crossing and repeated selection, such as have been described and explained at length by LEVAN (1945 a, b, 1948) and other authors.

While the hybrids were more fertile than either of the parents in some of the F_1 combinations in *L. amplexicaule*, *L. purpureum* and flax, as well as in the combination of vetch lines, this was far from the case in some other flax hybrids, nor in *Galeopsis pubescens* (F_2), nor perhaps in an occasional hybrid in *L. purpureum* where the seed fertility could

be raised only in subsequent generations. It will be realized, therefore, that a fertility improvement either may emerge immediately in the F_1 hybrids or become manifest only after repeated selection following hybridization. Clearly, then, we are dealing with two genetically different effects. When the F_1 hybrids have better seed setting than the parent lines, the effect is one that most closely approaches the phenomenon of heterosis. In such circumstances the improved fertility would seem to evince the interaction of more genes than the parents possessed; whereas the improved fertility after selection in hybrid offspring comes about from the interaction of genes that are more compatible with one another and with the tetraploid state than those in the pure lines. Should no selection whatever be made in the progeny of hybrids characterized by a higher fertility than the parent lines, the progeny probably would in due course differentiate into lines having dissimilar fertilities, which might be higher or lower than the F_1 generation's. Among various forms of heterosis, GUSTAFSSON (1951) distinguished a 'reproductive type', a term which evidently is applicable here. As no estimates were made of the vigour of F_1 hybrids in *Lamium*, it is impossible to say whether reproductive heterosis was at work alone or in concert with what GUSTAFSSON called 'the somatic type of heterosis'. These two expressions of heterosis are probably often present jointly, and it is likely that they were combined in *Lamium*, too: most of the hybrids appeared to be very vigorous and, being common in the corresponding diploid hybrids, heterosis was to be expected in the tetraploids (*cf.* HAGBERG, 1953).

Not only the seed setting but also the pollen fertility in hybrids of tetraploid *L. purpureum* showed a distinct tendency to exceed that in the pure lines. The pollen fertility thus fairly significantly exceeded the best parent line's in 3 of 7 hybrid combinations, notwithstanding the fact that many replicates comprised but few individuals. Apparently no equally marked effect of hybridization has been observed hitherto in autotetraploids — NORDENSKIÖLD (1953), admittedly, has examined the pollen fertility of hybrid offspring (F_6 or F_7?) in vetch and reported that she had found a very slight improvement, but the only data given seem to indicate the opposite ($\bar{x}$, as derived from the class mid-marks in Table 4, was 87,3 for the hybrids *versus* 88,8 and 89,0 for the respective parent lines). Conceivably the higher pollen fertility in the hybrids of *L. purpureum* might have resulted from the hybrids' having a more liberal supply of nutrients than the pure lines owing to the possible action of heterosis in the former, the improvement then merely being of modificatory nature. More likely, however, the improvement of the

pollen was dependent on genetic factors, just as the increased seed setting assumedly was. Accordingly the general presence of more genes than in the pure lines would make the pollen grains, though differentiated hereditarily through the segregation in F_1 and subsequent generations, more viable and better able to withstand the detrimental effects of aneuploid chromosome numbers and also of the genotypic unbalance characterizing tetraploids in general. As a matter of fact one would expect the pollen fertility of autotetraploids to improve following interlinear hybridization, just as the seed fertility and vigour on the whole can do under similar conditions.

2. ANEUPLOIDY.

The action of aneuploid chromosome numbers has thoroughly been studied in a great many species, *e. g.*, in *Datura*, *Matthiola*, *Lycopersicon*, *Zea*, tobacco, wheat, *etc.* The investigations have mainly been concerned with diploid or allopolyploid species, however; and the autopolyploids with their intrinsic peculiarities seem to have received a somewhat step-motherly treatment. Brief statements made in connection with other matters exist in respect of numerous species, but investigations devoted expressly to this subject are few indeed. Here the reader should recall the repeated statement to the effect that this study does not purport to be anything more than a survey of the subject.

Aneuploids occur in the progeny of the large majority of autotetraploids, but they may be more or less plentiful. Vetch seems to be an exception to this rule, for when examining altogether 317 tetraploid plants, NORDENSKIÖLD (1953) found not a single one with an aberrant chromosome number. Multivalents and univalents were fairly numerous, however, and the apparently total lack of aneuploids would seem to be merely a difference in degree from other species studied. As an example of the opposite extreme we may take tetraploid maize with 50 per cent of aneuploids which, admittedly, constituted the progeny resulting from selfing (RANDOLPH, 1935). As the majority of autotetraploids seem to have about 20 per cent of aneuploids, *L. amplexicaule* and *L. purpureum* with an average aneuploidy frequency of 37 and 34 per cent, respectively, lay well over the general run of autotetraploids. (It should be mentioned that the aneuploidy frequencies given here and in the following refer to offspring from euploid mother plants.)

Distinct interspecific differences obtain also with regard to the distribution of the aneuploid chromosome numbers about the normal value. In tetraploid garden varieties of *Petunia* 27 per cent were aneuploids,

and nearly two-thirds of these — namely 17 per cent of the total — were hypotetraploids (LEVAN, 1937). In *Dactylis glomerata* which, though strictly speaking no autotetraploid, resembles one in its cytological type, 6 per cent of the plants were hypotetraploids and 5 per cent hypertetraploids; the aneuploids of this species were thus equally distributed about the normal value (MÜNTZING, 1937; MYERS, 1947). In *L. amplexicaule* the hypertetraploids were on the average 5 times as numerous as the hypotetraploids. Practically as large a preponderance of hypertetraploids has been found also in *Galeopsis pubescens*, a species belonging to the same family as *Lamium* (MÜNTZING, 1941). With regard to hypertetraploid preponderance the latter species probably represent the extreme; more commonly they seem to be about twice as numerous as the hypotetraploids [maize, rye (MÜNTZING, 1951), *Lactuca sativa* (EINSET, 1947), *L. purpureum*], and predominance of hypertetraploidy appears on the whole more common than the reverse.

From many researches it is known that the gametes on the whole are more susceptible to chromosomal aberrations than the zygotes, and also that the pollen grains are far more susceptible than the ovules (*cf.* SANSOME and PHILP, 1939). Nor are unbalanced though vital ovules eliminated by certation, *etc.*, as are male gametes. Moreover, gametes and zygotes are in general more influenced by lack than by excess of one or more chromosomes. Probably the dissimilar aneuploid distributions about the normal values for the autotetraploid species are chiefly determined by the degree of susceptibility and of reduced vigour of, especially, the male gametes of various categories and also by the frequencies of each type of gamete. Owing to chromosomal elimination at meiosis, the mean gametic chromosome numbers of autotetraploids are presumably somewhat subnormal. In these circumstances, the very skew distribution of the aneuploids in *L. amplexicaule* must be attributed to a very strict elimination of hypotetraploid gametes and perhaps of zygotes, as MÜNTZING (1941) proposed for *Galeopsis pubescens*, and the chromosomal distribution from hypotetraploids bears out (p. 221). By way of comparison we may take, for example, tetraploid maize, where the hypertetraploid class percentages agree well with the corresponding groups in *L. amplexicaule*, but which has three times as many hypotetraploid plants and even includes spontaneous plants with $4x$—3 chromosomes. *Antirrhinum majus* apparently presents similar features (STRAUB, 1941).

The demonstrated means of raising the chromosome number variability by selection of extreme aneuploids may have a variety of causes.

Thus the aneuploid gametic frequency must be much greater in an aneuploid than in a euploid plant, and, in general, the more chromosomally aberrant the plant the greater will be the gametic chromosomal variability. Unlike the case of most diploids, aneuploid chromosome numbers have been shown to reduce only slightly the vigour of autotetraploids (see below). Presumably, therefore, diploid pollen grains with ± 1 aberrant chromosome are not as often lethal or non-functional as such haploid pollen grains of various species usually seem to be. Such an assumption is borne out by a number of investigations (*cf.* MÜNTZING, 1937). If so, the preponderance of male gametes with this type of aberration would, despite their very probably slower germination and pollen tube growth, give them better chances of fulfilling their reproductive role than their counterparts in euploid plants. And the viable female gametes especially will probably increase their chromosomal variability as a result of aneuploidy. Although the seed fertility probably would be reduced hereby owing to increased zygotic elimination, this might also together with the presumed greater variability of the viable pollen grains enable the numerical chromosome variability of the species to increase.

The on the whole slight influence of extremely aneuploid chromosome numbers on stem height and vigour in *Lamium* supports the assumption that gametic lethality (or gametic non-function) and/or elimination at early embryonic stages is the limiting factor for chromosomal variability. The reality of the second alternative gains credibility from MÜNTZING's (1943 c) finding that rye kernels with aneuploid chromosome numbers shrivel. Very weak *Lamium* plants, *e. g.*, those that failed to reach the flowering stage or otherwise departed markedly from normal, were thus completely lacking from the experiments. Nor was there any noticeable increase of the number of non-viable embryos after the seeds were husked (*cf.* BERNSTRÖM, 1952) — occasional such embryos will always occur. The hypotheses for control of chromosomal variation are borne out by, among other things, studies of chromosome number variability in *Beta* and other genera (LEVAN, 1942) and in *Dactylis* (MÜNTZING, 1937). However, particularly from the studies of *Dactylis* it appears also that hybridization of triploids and tetraploids is capable of expanding the chromosomal variation far beyond the bounds of spontaneous aneuploidy variation in the tetraploid, owing to the maximal chromosomal variation of gametes from triploids; though the fact that they involved hybrids of two separate but closely related species detracts somewhat from the value of these studies. This increment has

the same causes as were assumed to be operative in *Lamium*. In other words, a larger assortment of chromosomally varied gametes than normally is present would have occasion to produce a large variety of zygotes, some of which would survive and develop in spite of extreme unbalance.

This means that the more aberrant the chromosome number of a zygote, the greater will be the influence of the particular type of aberrant chromosome complement on the zygote's chances of surviving. Hence, the more discrepant the somatic chromosome numbers, the more difficult of realization will be those chromosomal combinations which marked abnormality notwithstanding are capable of forming the bare units essential to the vital processes. Since, as mentioned, the progeny of extremely aneuploid plants always can develop into acceptably vigorous and profusely flowering plants, the elimination of non-vital zygotes must, when it occurs, take place at an early stage in the development of the zygote. The significance of particular chromosomes for the viability of gametes and zygotes with aneuploid chromosome numbers has been demonstrated in, especially, *Datura* by BLAKESLEE and coworkers and in autopolyploid material of *Dactylis* by MÜNTZING (1937). Mode of meiosis combined with the particular chromosomes involved has been shown by these authors to affect the chromosome number distribution of progenies of aneuploid plants. Obviously, analogous processes in the two *Lamium* species are responsible for the differences in chromosome number distribution in different progenies (see above), but the present investigation could not be made so detailed and the material was more complex.

With regard to the influence of aberrant chromosome numbers on various characters, a general experience of autotetraploids of different species seems to be that chromosomal unbalance only slightly affects the vigour. No marked association between deficient vigour and aberrant chromosome numbers has thus been found in, for example, tetraploid maize (RANDOLPH, 1935), *Antirrhinum majus* (STRAUB, 1941) and *Lactuca sativa* (EINSET, 1947). Such an association is probably present in *Galeopsis pubescens* and tetraploid rye (MÜNTZING, 1941, 1951), and it was definitely the case in *L. amplexicaule* and *L. purpureum*, particularly in the latter. In *L. amplexicaule*, on the other hand, florescence was found to occur later in the aneuploids than in the chromosomally normal plants, whereas no such thing could be observed in *L. purpureum*. In view of the narrow scope, or preliminary nature, of the studies cited above, the absence of a definite association between chro-

mosome number and plant vigour in several species must probably be interpreted to mean that the association has the same low level of significance as in the *Lamium* species. It is highly improbable that aberrant chromosome numbers in any autotetraploid species would reduce the plant vigour not at all; but, conversely, one may expect aneuploidy to be much less apparent in autotetraploids than in diploids, because the aberrations are proportionately smaller in the former. A contributory cause of the comparatively favourable vigour and stem height in *Lamium* aneuploids might be — though it makes no claims to credibility — that gradually increasing sterility with rising aneuploidy is associated with an inversely proportional vigour. But this hypothesis is contradicted by the fairly undeficient vigour of other aneuploids of autotetraploids that have been studied, *e.g.*, rye and maize, in which sterility-promoted growth seems most unlikely.

In this connection attention may be drawn to the late florescence of *L. amplexicaule*, particularly the delay and deficiency of the chasmogamous flowering. In a preceding publication the old observation has been recalled that deficient nutrition tends to make the flowering of *L. amplexicaule* predominantly cleistogamous (BERNSTRÖM, 1950 b), and the observation was repeated in many of these experiments. As the chasmogamous flowering of the aneuploids in *L. amplexicaule* is late and sparse compared with the chromosomally normal plants, this may be a consequence of impaired physiological balance in the aneuploids which affects the flowering of the plants in the same way as more or less deficient nutrition. The latter, then, would sooner affect the type of flowers than the vigour of the plants.

The fertility of aneuploid autotetraploids seems to have attracted relatively little attention. However, combined with experiences in *Lamium*, what little has been published suggests that the fertility undergoes a considerable reduction. The pollen fertility of *Lactuca sativa* plants with $4x-1$, $4x+1$ and $4x+2$ was slightly lower than in chromosomally normal individuals, whereas all categories showed a sharp seed-setting decline (EINSET, 1947). In *Galeopsis pubescens*, MÜNTZING (1941) found that the aneuploids had about 80 per cent of the euploids' pollen fertility. Studying *Dactylis glomerata*, which probably for the purposes at hand closely resembles true autotetraploids (see above), the latter author (1937) noted that the pollen fertility ratio between the aneuploids and the euploids was about the same as in the *Lamium* species studied here; in addition the seed setting of the aneuploids was much lower.

The above reasoning partly explains the reduced fertility of aneuploids. Accordingly the lower pollen fertility must in the first place be attributed to the more irregular meiosis in aneuploids, so that more gametes with aneuploid chromosome numbers are formed than is the case in euploid plants. Presumably, however, a factor contributing to the fertility reduction is a general weakening of the pollen produced by the aneuploids' worse genotypic balance, thus introducing also a diplontic element into the sterility. Besides, in accordance with previous statements on the pollen fertility, estimated by germination experiments in autotetraploids, it is very likely that its gradual reduction with increasing chromosomal aberration in aneuploids is much greater than pollen analyses would lead one to suppose.

The seed fertility of the aneuploids may, too, be assumed to undergo various adverse changes as a result of a greater genotypic unbalance than autopolyploids characteristically have. In a line of autotetraploid *Lactuca sativa*, EINSET (1947) found a significant preponderance of undeveloped embryo sacs among the aneuploids; and, though not the case in another line, this was, in the line concerned, most likely the result of increased genic unbalance. Other evidences of diplontic sterility in aneuploids have been given by MÜNTZING (1937). In the *Lamium* species, it was repeatedly observed that a large proportion of the flowers of obviously aneuploid plants had anthers and style which in various ways were singularly ill adapted to one another, so that reduced seed setting would follow. An analogy to defective pollination which must contribute something to the seed-setting reduction is the lesser availability of functional gametes in aneuploids due to the higher frequency of gametic unbalance. Lastly, if the above supposition of inclining zygotic elimination at embryonic stages is a reality, it, too, must serve to reduce the seed setting.

To sum up, then, it may be said that although most of the above as yet is hypothetical rather than factual it does, all things considered, furnish a fully adequate explanation of the markedly reduced fertility of the aneuploids.

SUMMARY.

(1) Several lines of each of the species *Lamium amplexicaule* and *L. purpureum* have been studied. The general properties of the autotetraploids of these species have been outlined briefly.

(2) The pollen fertility of the tetraploids of both species ranged from 85 to 98 per cent of the values for the corresponding diploids. In

L. purpureum 3 of the 7 interlinear hybrid combinations studied the pollen fertility was obviously superior to the parents', most probably because the greater gene mass (*i. e.* the sum of the parental gene complements) was more compatible with the tetraploid »state».

(3) In *L. amplexicaule* the seed setting of the tetraploids was about 65 per cent of that of the diploids, while the corresponding relation in *L. purpureum* was about 50 per cent. The effects of environment on the seed-setting degree in pure lines of both species is discussed. The seed setting could easily be improved in both species by interlinear hybridization, F_1 hybrids not seldom having a seed setting superior to the best of the respective parent lines. The cause of this is assumed to be a heterosis-like effect.

(4) Both species had approximately 6 multivalents per cell, as compared with a possible maximum of 9. No detailed cytological investigations could be carried out.

(5) Both species displayed a comparatively high frequency of aneuploids (25—43 %). Probable aneuploid frequency differences were found between lines of *L. amplexicaule*. These differences are ascribed to the fact that the lines had dissimilar proportions of cleistogamous flowers, which presumably promote the formation of aneuploids. The spontaneous chromosome number variability was in both species characterized by a distinctly skew distribution (range in *L. amplexicaule*: 35—39; in *L. purpureum*: 35—38). The range of variability could without difficulty be doubled in both species by selection of extreme aneuploids (range: 34—42 or 43 chromosomes). Significant differences in chromosome number distribution were found between some offspring of plants with the same aneuploid chromosome number, depending on the chromosomal constitution of the mother plants.

(6) In both species (1 line of each studied) there was a strong positive correlation between declining seed setting and inclining chromosome number aberration. The loss of one chromosome was just as deleterious as the acquisition of two. In principle the same was true of the pollen fertility; but, relatively, this was not reduced as much as the seed fertility. Stem height and vigour underwent a gradual but steady reduction as the degree of aneuploidy increased, more so in *L. purpureum* than in *L. amplexicaule*. In *L. amplexicaule* the number of leaf indentations declined similarly. While the same was true for the time of cleistogamous and chasmogamous florescence (the greater the chromosomal aberration the longer the delay) in *L. amplexicaule*, no such

correlation between florescence and chromosome number was observed in *L. purpureum*.

(7) The fertility reduction of the tetraploids is discussed. In addition to the consequences for gametic and zygotic viability of the irregular meiosis, a number of other manifestations are mentioned, all of which originate from the upset genotypic balance of the tetraploids. Attention is thus drawn to various causes of inhibited pollination, to fertilization failure despite pollination owing to various disturbances in the fertilization process, to low true pollen fertility, *etc.*; the effects of the concerted action of these factors are dealt with.

(8) Aneuploids have different frequencies and are dissimilarly distributed in autotetraploids of various species. These interspecific differences are in the first place ascribed to varying degrees of elimination of chromosomally unbalanced gametes and new zygotes. Factors promoting, on the one hand, and factors inhibiting, on the other, an expansion of the intraspecific chromosome number variability when aneuploids are selected are discussed in the light of the facts governing spontaneous aneuploidy variation. In this connection the effect of aneuploid chromosome numbers on, particularly, the fertility is analysed.

LITERATURE CITED.

1. BERNSTRÖM, P. 1949. Cytogenetic studies in the genus *Lamium*. — Proc. 8th Int. Congr. Genetics (Hereditas, Suppl. Vol.).
2. — 1950 a. Does the fertility of intraspecific hybrids in autogamous species improve in consequence of a heterotic effect? — Hereditas XXXVI.
3. — 1950 b. Cleisto- and chasmogamic seed setting in di- and tetraploid *Lamium amplexicaule*. — Hereditas XXXVI.
4. — 1952. Cytogenetic intraspecific studies in *Lamium*. I. — Hereditas XXXVIII.
5. — 1953 a. Increased crossability in *Lamium* after chromosome doubling. — Hereditas XXXIX (Festskrift för ARNE MÜNTZING 2.3.1953).
6. — 1953 b. Cytogenetic intraspecific studies in *Lamium*. II. — Hereditas XXXIX.
7. COOPER, D. C. and BRINK, R. A. 1945. Seed collapse following matings between diploid and tetraploid races of *Lycopersicon pimpinellifolium*. — Genetics 30.
8. DARLINGTON, C. D. 1937. Recent advances in cytology. 2nd ed. — London.
9. EINSET, J. 1944. Cytological basis for sterility in induced autotetraploid lettuce (*Lactuca sativa* L.). — Amer. Journ. Bot. 31.

9a. — 1947. Aneuploidy in relation to partial sterility in autotetraploid lettuce (*Lactuca sativa* L.). — Amer. Journ. Bot. 34.

10. FISHER, R. A. 1938. Statistical methods for research workers. 7th ed. — London.
11. GUSTAFSSON, Å. 1951. Mutations, environment and evolution. — Cold Spring Harbor Symp. Quant. Biol. 16.

12. HAGBERG, A. 1953. Further studies on and discussion of the heterosis phenomenon. — Hereditas XXXIX.
13. HÅKANSSON, A. and ELLERSTRÖM, S. 1950. Seed development after reciprocal crosses between diploid and tetraploid rye. — Hereditas XXXVI.
14. KOKHANOVSKAYA, L. N. 1939. On the physiological conditions of pollen germination in flax. — C. R. Acad. Sci. U.R.S.S. 24.
15. KUCKUCK, H. und LEVAN, A. 1951. Vergleichende Untersuchungen an diploiden und tetraploiden Leinsippen und an tetraploiden Kreuzungsnachkommenschaften nach vieljähriger Selektion. — Der Züchter 21.
16. LAMM, R. 1944. Chromosome behaviour in a tetraploid rye plant. — Hereditas XXX.
17. LEVAN, A. 1937. Chromosome numbers in *Petunia*. — Hereditas XXIII.
18. — 1942. The effect of chromosomal variation in sugar beets. — Hereditas XXVIII.
19. — 1945 a. Aktuelle Probleme der Polyploidiezüchtung. — Arch. Julius Klaus-Stiftung, Erg.-bd. zu Bd. 20.
20. — 1945 b. Polyploidiförädlingens nuvarande läge (with an English summary). — Sv. Utsädesförenings Tidskr. 1945.
21. — 1948. The Cyto-genetic Department 1931—1947. Svalöf 1886—1946. — Lund 1948.
22. MYERS, W. M. 1947. Studies on the origin of *Dactylis glomerata* L. — Records Gen. Soc. America 16.
23. MÜNTZING, A. 1936. The evolutionary significance of autopolyploidy. — Hereditas XXI.
24. — 1937. The effects of chromosomal variation in *Dactylis*. — Hereditas XXIII.
25. — 1941. New material and cross combinations in *Galeopsis* after colchicine-induced chromosome doubling. — Hereditas XXVII.
26. — 1943 a. Fertility improvement by recombination in autotetraploid *Galeopsis pubescens*. — Hereditas XXIX.
27. — 1943 b. Några försöksresultat med rågvete och tetraploitt korn. — Nord. Jordbrugsforskning 25.
28. — 1943 c. Aneuploidy and seed shrivelling in tetraploid rye. — Hereditas XXIX.
29. — 1951. Cyto-genetic properties and practical value of tetraploid rye. — Hereditas XXXVII.
30. NORDENSKIÖLD, H. 1953. An investigation into two tetraploid strains of vetches (*Vicia sativa*) and their hybrid products. — K. Lantbrukshögskolans Annaler 19.
31. PIRSCHLE, K. 1942. Quantitative Untersuchungen über Wachstum und »Ertrag» autopolyploider Pflanzen. — Zschr. f. ind. Abst.- u. Vererbl. 80.
32. RANDOLPH, L. F. 1935. Cytogenetics of tetraploid maize. — Journal Agric. Research 50.
33. SANSOME, F. W. and PHILP, J. 1939. Recent advances in plant genetics. — London.
34. SCHWANITZ, F. 1942. Über die Pollenkeimung einiger diploider Pflanzen und ihrer Autotetraploiden in künstlichen Medien. — Der Züchter 14.
35. — 1950. Untersuchungen an polyploiden Pflanzen. VI. Pollengrösse und Zellkerngrösse bei diploiden und autotetraploiden Pflanzen. — Der Züchter 20.
36. SCHWANITZ, F. und H. 1950. Untersuchungen an polyploiden Pflanzen. X. Weitere Beiträge zur Sexualität polyploider Pflanzen. — Der Züchter 20.

37. STRAUB, J. 1941. Die Nachkommenschaft des tetraploiden *Antirrhinum majus* Sippe 50. — Ber. Deutsch. Bot. Ges. 59.

38. v. WOESS, F. 1941. Experimentelle Untersuchungen zum Artbildungsproblem an *Arenaria serpyllifolia* und *Arenaria Marschlinsii*. — Zschr. f. ind. Abst.- u. Vererbl. 79.

CONTENTS.

I. Introduction 181
II. Materials, methods and general characters of the autotetraploids 182
III. Experimental 186
1. *L. amplexicaule* 186
A. Fertility 186
B. Cytology 190
C. Aneuploidy 194
a. Chromosomal variation 194
b. Effects of chromosomal variation 199
2. *L. purpureum* 207
A. Fertility 207
B. Cytology 215
C. Aneuploidy 216
a. Chromosomal variation 216
b. Effects of chromosomal variation 220
IV. Discussion 227
1. Fertility 227
2. Aneuploidy 232
Summary 237
Literature cited 239

SOME OBSERVATIONS ON P^{32} CONCENTRATION AND DISTRIBUTION IN LILIUM BUDS

BY *W. S. PLAUT*
INSTITUTE OF GENETICS, LUND

THE use of radioactive isotopes in cytological research, as well as in the more usual physiological and biochemical studies, generally presupposes that the level of tracer activity is kept well below the point where radiation damage becomes a complicating factor. The readiness with which this prerequisite can be fulfilled depends largely on the radiosensitivity of the material or process under investigation and the methods employed for administering the isotope and detecting its final distribution or concentration. That extreme caution is necessary in the establishment of a safe level of activity, in the selection of valid criteria for ascertaining the absence of radiation damage, has been very succinctly pointed out by RUSSELL (1953) in the recent past (see also EHRENBERG and GRANHALL, 1952).

In this connection I should like to call attention to some data collected while studying the uptake rate, distribution, and concentration of P^{32} in the buds of *Lilium Henryi* and *L. longiflorum*. The study was directed toward the modification of a previously used technique (PLAUT, 1953) and obtaining some critical data on the duration of DNA synthesis in the preleptotene microsporocytes. Cut inflorescences were placed in solutions consisting of distilled water, 18,5 μg Na_2HPO_4 per cc as carrier, and generally 16 μC P^{32} per cc (obtained as inorganic phosphate from Harwell). In some cases P^{32} activities of 4, 9, 20, and 80 μC per cc were used. The pH of the solutions was unfortunately not determined for each culture; subsequent measurements indicated a probable pH value of about 6 for these solutions. The activity of one bud on each inflorescence was sampled at frequent intervals with a Geiger-Mueller tube connected to a standard scaling instrument. The counting tube was heavily shielded to exclude all radiation except that emanating from a small surface fraction of the buds (12,6 mm^2). Other buds were removed from the inflorescences at different times; two of their anthers were weighed and set aside for an analysis of their P^{32} content, and the re-

TABLE 1. *P^{32} activity in anthers (μC P^{32}/gram tissue).*[1]

Hours of Immersion of Parent Inflorescence	*L. Henryi*				*L. longiflorum*		
	Solution Activity μC P^{32}/cc				Solution Activity μC P^{32}/cc		
	4	9	16	80	9	16	20
2						0,2(1)	
3							0,3(1)
4						0,3(2)	
5				0,8(1)			
6							1,1(1)
7						2,2(1)	
8				13,4(1)			
9			1,0(3)			1,2(1)	
10			1,4(1)				
11			1,0(2)				
12			1,6(2)			1,6(1)	
13		6,3(1)	4,4(2)		2,4(1)		
14	2,5(1)	5,9(1)	2,4(3)	66,2(1)			
15			5,2(2)				
17			3,5(2)			5,2(1)	
18	7,8(1)		6,5(2)				
22			9,1(4)				
26	19,1(1)	13,1(1)					
37			15,9(2)				

[1] The figures are based on the averages of two anthers per bud. The numbers in brackets indicate the number of anther pairs from buds on different inflorescences averaged for the respective value.

maining anthers fixed and processed into autoradiograph slides. A few buds were dissected into anther, pistil, and outer tissue fractions for subsequent analysis of the P^{32} distribution within the bud.

The results of the anther P^{32} analysis are summarized in Table 1. Although the variability is too great to permit the prediction of an activity level in the anthers of one bud from measurements in another (*cf.* GILES and BOLOMEY, 1948), to say nothing of a prediction on the basis of the specific activity of the solution in which the parent inflorescence was immersed, the order of magnitude of the P^{32} activities in the anthers is clearly indicated and alarmingly high. (It should be

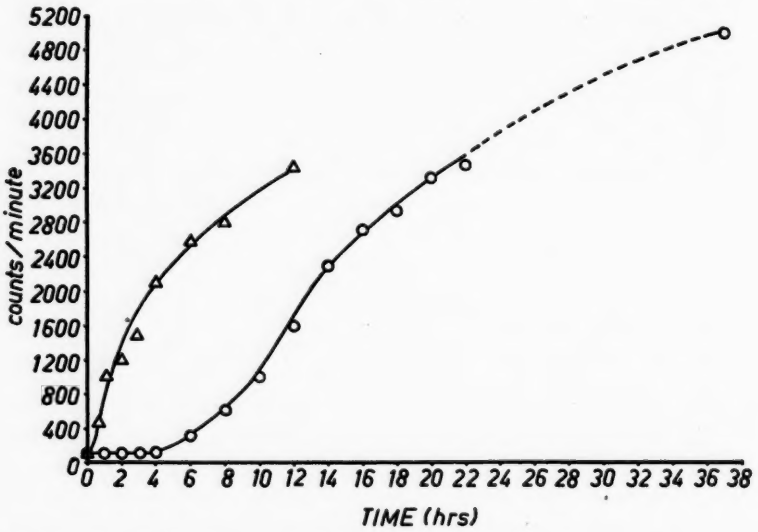


Fig. 1. Activity in *Lilium Henryi* and *L. longiflorum* buds on inflorescences immersed in solutions containing 16 μC P^{32} per ml and carrier. The plotted points (○=*L. Henryi*, △ =*L. longiflorum*) represent averages of two buds, one on each of two inflorescences.

pointed out that the figures given are subject to an error of 10 % or less since the P^{32} determinations were made without an absolute standard.)

When bud surface activity values (counts per minute) recorded with the G.-M. counter for a series of *L. Henryi* buds were compared with similarly obtained *L. longiflorum* data, it was noted that the two curves which were derived differed sharply over the first few hours of exposure of the inflorescence to the active solutions. (See Fig. 1 for two typical curves.) The *L. Henryi* buds appear to remain at background level for 4 to 5 hours while those of *L. longiflorum* show a rise in activity within less than 10 minutes. Although there are some structural differences between the two species which may affect the time required for the P^{32} solution to reach the buds, the main reason for the difference between the two curves seems to arise from dissimilarities in the distribution of P^{32} among the various tissues of the buds (see Table 2 for the results of whole-bud analyses).

P^{32} beta particles have a range in tissue of about 3 mm. Most, if not all of the »counts» detected at the surface of a bud emanate from decaying P^{32} atoms within the outer tissues of the bud; very little, if

anything, is contributed by P^{32} in the anthers or in the pistil. Thus, while the total amounts of P^{32} in the buds may be similar, the *L. Henryi* bud will manifest a considerably smaller fraction of the isotope at the bud surface than the *L. longiflorum* bud. The values of Table 2 seem to point to a change of the ratio of anther to outer tissue P^{32} content with time of immersion of the inflorescence in the active solution; the data are not extensive enough, however, to warrant generalization. One conclusion which can be drawn is that *a priori* assumptions concerning the P^{32} distribution within buds and possible similarities in this distribution pattern between related species are not justified.

The autoradiograph preparations have been scanned in the hope of discovering a consistent relationship between time in active solution and/or activity in anthers, and the presence of nuclear autoradiographs. So far no simple relationship has been observed. This need not necessarily indicate serious errors in the P^{32} content values since the anthers used for autoradiograph preparations differ from those analysed for P^{32} content in that their phospholipids, RNA, and most other carriers of non-DNA phosphorus are removed before the tissues are brought into contact with the photographic emulsion. Only a nucleus incorporating phosphorus into DNA when P^{32} is available will be detected by an autoradiograph. The minimal number of P^{32} atoms necessary to make a nucleus »detectable» could be acquired in a short time with a relatively high P^{32}/P^{31} ratio, or in a longer time when a lower ratio prevails. The presence of autoradiographs is thus dependent on two interacting environmental factors, time and P^{32}/P^{31} ratio, and one intrinsic one, the presence of synthesizing nuclei. The data are at present far from sufficient to permit the formulation of a general relationship which will take these complexities into account.

The primary interest in these diverse observations, and the reason for their publication at this time, lies in the high P^{32} activities found in the anthers, where the synthetic process to be studied takes place. By applying a simple formula to these values, an approximate radiation dose rate can be calculated. [$\text{Reps/hour} = 60 \times C \times E_{av}/24$, where C denotes tissue activity in μC/gram, and E_{av} the average particle energy in MeV, 0,7 for P^{32}; the hourly dose is obtained by multiplying the μC/gram value by 1,75 $(60 \times 0{,}7/24)$; the formula is given by KAMEN, 1951.] Sample dose rates for 2, 10, 50 μC/gram are, respectively, 3,5, 17,5, and 87,5 reps per hour. It is evident that if such rates prevail for even a few hours the total dose received by the anther tissues falls into the range where radiation damage must be considered.

TABLE 2. *Distribution of P^{32} within buds.*

Inflorescence Number	Hours in Solution	Soln. Activity μC P^{32}/cc	Bud Part	Activity μC P^{32}/g	Anther Activity / Outer Tissue Activity
L. longifl. 4	7	16	Anthers [1]	2,2	
			Pistil	0,7	1,1
			Outer Tissue	1,9	
L. longifl. 5	17	16	Anthers	5,2	
			Pistil	0,9	4,4
			Outer Tissue	1,2	
L. Henryi 14	18	16	Anthers	4,1	
			Pistil	0,5	15,3
			Outer Tissue	0,3	
L. Henryi 15 (1st bud)	18	16	Anthers	8,9	
			Pistil	1,9	13,1
			Outer Tissue	0,7	
L. Henryi 15 (2nd bud)	37	16	Anthers	14,7	
			Pistil	2,6	9,9
			Outer Tissue	1,5	

[1] The anther activity values are based on samples of two anthers per bud. The weights used in the calculation of specific tissue activities were fresh weights.

GRAY and SCHOLES (1951) found that a dose of 20 r of X-rays was sufficient, if delivered to the meristematic region, to affect measurably the growth rate of *Vicia faba* roots. RUSSELL (1953) has pointed out that while some barley seedlings suffer physiological damage with 20 to 50 r of P^{32} beta irradiation, others appear undamaged by doses of over 200 r. HAQUE (1953) showed that doses of 12 and 18 r of X-rays will produce cytological irregularities in meiosis of *Tradescantia*.

In addition, there is a strong probability that DNA synthesis itself is affected by X-rays, at least in so far as mitotic cells are concerned. This has been demonstrated in animal tissue (see review by HEVESY, 1952), and in *Vicia faba* (HOWARD and PELC, 1953). Although SPARROW, MOSES, and STEELE (1952) have concluded that there is no apparent relationship between DNA content of the microsporocytes and X-ray sensitivity in *Trillium*, nothing is known about the sensitivity of the synthetic process in meiosis, or, for that matter, about the comparability of P^{32} beta irradiation and X-rays in their effect on DNA synthesis *per se*.

In the analysis of autoradiographic data of DNA synthesis in the microsporocytes the cytologist's normal criteria for radiation damage (*i. e.* physical chromosome disturbances, spindle mechanism effects) are of little use as the dose relationships between such disturbances and possible DNA synthesis abnormalities are not known. Until such information becomes available and possible effects of P^{32} beta irradiation on meiotic DNA synthesis can be either excluded or critically assessed, data obtained with P^{32} autoradiography must be interpreted without the assumption that the incorporation process leading to the autoradiograph continued undisturbed in the presence of the isotope. All that can be stated is that a nucleus at a given stage of development did or did not incorporate sufficient P^{32} into its DNA to make it detectable by an autoradiograph.

Acknowledgements. — The work reported was done while the author was a U.S. Public Health Research Fellow of the National Cancer Institute. I should also like to express my gratitude to colleagues at the Institutet för Biokemi, Stockholm, particularly to Mrs. M. BRUNNBERG, for carrying out the P^{32} content analyses, and to Drs. LEVAN and MÜNTZING of the Genetics Institute, Lund, for generously providing me with the necessary equipment and facilities.

SUMMARY.

Some observations made on the uptake rate, concentration, and distribution of P^{32} in *Lilium* buds are reported. Attention is directed to the relatively high P^{32} activities obtained in the anthers. The implication of the resultant radiation dosages with respect to the validity of conclusions drawn from autoradiographic studies of these tissues is briefly discussed.

LITERATURE CITED.

1. EHRENBERG, L. and GRANHALL, I. 1952. Effects of beta-radiating isotopes in fruit trees. — Hereditas XXXVIII: 385—419.
2. GILES, N. H. JR. and BOLOMEY, R. A. 1948. Cytogenetical effects of internal radiation from radioisotopes. — Cold Spring Harb. Symp. Quant. Biol. XII: 104—112.
3. GRAY, L. H. and SCHOLES, M. E. 1951. The effect of ionizing radiations on the broad bean root. VIII. Growth rate studies and histological analyses. — British Journ. Radiology 24: 82, 176, 228, 285, 348.

4. HAQUE, A. 1953. The irradiation of meiosis in *Tradescantia*. — Symp. on Chromosome Breakage; Suppl. to Heredity 6: 57—75.
5. HEVESY, G. 1952. Ionizing radiation and cellular metabolism. — Symp. on Radiobiology (Oberlin College): 189—215. (John Wiley: New York and London.)
6. HOWARD, A. and PELC, S. R. 1953. Synthesis of desoxyribonucleic acid in normal and irradiated cells and its relation to chromosome breakage. — Symp. on Chromosome Breakage; Suppl. to Heredity 6: 261—273.
7. KAMEN, M. D. 1951. Radioactive Tracers in Biology. — Academic Press Inc.; New York.
8. PLAUT, W. S. 1953. DNA synthesis in the microsporocytes of *Lilium Henryi*. — Hereditas XXXIX: 438—444.
9. RUSSELL, R. S. 1953. Problems in the use of phosphorus 32 in the study of plant nutrition. — Radioisotope Techniques. I. (Medical and Physiological Applications): 402—411. (H. M. Stationery Office: London.)
10. SPARROW, A. H., MOSES, M. J. and STEELE, R. 1952. A cytological approach to an understanding of radiation damage in dividing cells. — British Journ. Radiology 25: 182—189.

ABSTRACTS

GÖSTA OLSSON: Crosses between *Brassica napus* L. and Japanese *Brassica napella* CHAIX.

In several theoretical investigations of species belonging to the genus *Brassica* Japanese authors have used a "species" *Brassica napella* CHAIX. According to MORINAGA (1928, 1929 b), the chromosome number of *napella* is $n=19$, *i. e.* the same as in *B. napus* L. (NAGAI and SASAOKA, 1930). MORINAGA (1929 b) also states that *B. napella* "externally resembles *B. napus* very closely, which fact suggests strongly its origin in that species", and in a paper dealing with haploid *napella* MORINAGA and FUKUSHIMA (1933) state in a foot-note that "some workers treat this plant under the specific name *Brassica napus*". On the other hand, SCHULZ (1919) quotes *B. napella* VILL. as synonymous to *B. campestris* L. var. *autumnalis* DC., while HEGI considers *B. napella* CHAIX ap. VILL. as synonymous to *B. rapa* L. var. *silvestris* (LAM.) PURCHAS et LEY.

MORINAGA (1929a) studied crosses between *B. napella* and different *Brassica* species with the haploid chromosome number $n=10$ (*B. rapa, B. chinensis* and others) and found F_1 of these hybrids to be partly sterile, forming 10_{II} and 9_I in M_I. The aim of the present study is an investigation of the relation between *B. napus* and *B. napella*. For this purpose the two species have been crossed and fertility and heterosis studied in F_1. (The name *B. napella* is here used for the Japanese material obtained under this name, although, as will be shown later on, the name is most probably incorrect.)

The material used of *B. napella* has been obtained from Professor MIZUSHIMA, Sendai, Japan. When sending the seed, MIZUSHIMA also offered the information that this material of *B. napella*, the local name of which is "Wase Chose", "was introduced into Japan through Korean Peninsula, but supposed not to be of Korean origin. The seeds I am sending you are those of an improved strain selected at Konosu which is characterized to be dwarf, early maturing and very productive". According to MORINAGA (1928), *B. napella* is "widely cultivated for oil in southern Japan". — *B. napus* has been represented by the Swedish variety Svalöf's Regina spring rape.

Morphologically the two species are very much alike, but *B. napella* is distinctly different from Regina as well as from other European varieties of *napus* grown in our material, its rosette-leaves being more rounded and having less incised margins and the lower leaves on its stalk being nearly opposite. The variety of *napella* used flowers and ripens considerably later than Regina.

In crosses between the species the following results have been obtained:

	Number of flowers pollinated	Number of seeds per flower
B. napus × *B. napus*	107	13,9
B. napus × *B. napella*	56	11,4
Reciprocal	60	10,3

The cross between *napus* and *napella* gives just about as good results as that between different varieties of *napus*. The hybrid is morphologically intermediate and, as seen from Tables 1 and 2, fully fertile.

TABLE 1. *Pollen fertility in F1 of the cross B. napus × B. napella.*

Material	Percentage good pollen 60	65	70	75	80	85	90	95	100	n	M
B. napus				1				5	94	100	97,0
B. napus×*B. napella*				1	1	1	1	11	81	96	96,2
Reciprocal						1	1	26	68	96	95,9
B. napella		1			1	1	2	11	84	100	96,1

TABLE 2. *Seed fertility in F_1 of the cross B. napus × B. napella.*

Material	Percentage of well developed seeds <50	55	60	65	70	75	80	85	90	95	100	n	M
B. napus			1	1		4	10	13	31	29	13	102	87,5
B. napus×*B. napella*		1	2		1	2	2	19	25	35	9	96	87,6
Reciprocal				2	3	4	12	17	19	25	14	96	86,4
B. napella	2	1	4	3	9	14	8	20	13	13	4	92	78,5

TABLE 3. *Plant height in F1 of the cross B. napus × B. napella.*

Material	n	Plant height in cm M ± m
1. *B. napus*	102	109,6 ± 1,24
2. *B. napus*×*B. napella*	96	116,8 ± 1,49
3. Reciprocal	96	119,5 ± 1,37
4. *B. napella*	92	94,1 ± 1,20

t-value for diff. 2 — 1 = 3,71***; t-value for diff. 3 — 2 = 1,34°.

TABLE 4. *Seed weight per plant in F1 of the cross B. napus × B. napella.*

Material	n	Seed weight g/plant M ± m
1. *B. napus*	102	6,81 ± 0,44
2. *B. napus*×*B. napella*	96	7,82 ± 0,62
3. Reciprocal	96	7,53 ± 0,48
4. *B. napella*	92	2,99 ± 0,24

t-value for diff. 2 — 1 = 1,33°.

In order to obtain preliminary information in regard to heterosis, plant height and seed weight per plant have been determined. As seen from Tables 3 and 4, the *F*1 plants are taller and have a higher yield of seed than both parents. Only in regard to plant height there is, however, a statistically significant heterosis, if this term is taken as denoting superiority over the best parent (*cf.* HAGBERG, 1952). It is hardly surprising that there is no significant superiority in seed yield, to the best of the parents, as the other one has a very low yield. As a complement to the data in Table 4 it may be mentioned that in a preliminary yield test in 1950 the yield of *napella* was only 33 % of that of Regina. The low seed yield of *napella* is probably, at least partly, caused by the late flowering and ripening.

The results of the Japanese investigators quoted, as well as those from the crosses here reported, indicate that the "*napella*" used in these cases, besides being morphologically similar to *B. napus*, is easily crossed with this species,

giving a fertile hybrid; evidently it should be classified as a variety of *B. napus*. To denote it as a separate species, under the name of *B. napella* CHAIX, as has been done by NAKAI when determining the material of MORINAGA, is hardly correct, especially since the description of VILLARS (1786, 1789) of *B. napella* CHAIX concerns the spring and winter forms of turnip rape, *i. e.* a species with $n=10$ and not with $n=19$ chromosomes. The classifications of SCHULZ and HEGI refer to the descriptions of VILLARS, and in the opinion of the present author *B. napella* CHAIX is to be considered as a synonym for *B. campestris* L. and not used on material belonging to *B. napus* L.

It is impossible at present to know whether the East Asiatic material of *B. napus* has been introduced from the Mediterranean region where the species is supposed to have originated (SINSKAJA, 1928), or whether it has originated directly in eastern Asia. As shown by U (1935), FRANDSEN (1947), RUDORF (1950) and the present author (unpubl.), *B. napus* is obtained from crosses between *B. campestris* and *B. oleracea*, and most probably the species has appeared in nature as a result of identical phenomena. It is conceivable that the whole of the existing species *B. napus* s. lat. is derived from one single such cross and that the present differentiation is due only to mutation and selection; it seems more probable, however, that the cross has occurred several times in different localities, different types of the parental species having taken part at the different occasions. Varieties belonging to *B. oleracea* s. lat. occur in western Europe, the Mediterranean region and in eastern Asia, while *B. campestris* s. lat. is spread over the whole area, from western Europe to eastern Asia. Varieties of *napus* may therefore conceivably have been originated in eastern Asia as well as in Europe, and it is not unreasonable to accept the hypothesis that the Japanese material here studied represents a *napus* variety of East Asiatic origin. Even if this should be the case, there is no reason to treat it as a separate species. Amphidiploids between *B. oleracea* and *B. campestris* with $2n=38$, which are morphologically similar and which intercross easily, forming a fertile F_1, should all be considered as belonging to *B. napus*, even if they have originated at widely different times and places.

Literature cited.

1. FRANDSEN, K. J. 1947. The experimental formation of *Brassica napus* L. var. *oleifera* DC. and *Brassica carinata* BRAUN. — Dansk Bot. Arkiv 12(7): 1—16.
2. HAGBERG, A. 1952. Heterosis in F_1 combinations in *Galeopsis*. I and II. — Hereditas XXXVIII: 33—82 and 221—245.
3. HEGI, G. Illustrierte Flora von Mittel-Europa. IV, 1: 259. — München.
4. MORINAGA, T. 1928. Preliminary note on interspecific hybridization in *Brassica*. — Proc. of Imp. Acad. 4: 620—622.
5. — 1929 a. Interspecific hybridization in *Brassica*. I. The cytology of F_1 hybrids of *B. Napella* and various other species with 10 chromosomes. — Cytologia 1: 16—27.
6. — 1929 b. Interspecific hybridization in *Brassica*. III. The cytology of F_1 hybrid of *B. cernua* and *B. Napella*. — Journ. Dept. Agr. Kyushu Imp. Univ. 2: 199—206.

7. MORINAGA, T. and FUKUSHIMA, E. 1933. Karyological studies on a spontaneous haploid mutant of *Brassica Napella*. — Cytologia 4: 457—460.

8. NAGAI, K. and SASAOKA, T. 1930. The number of chromosomes in cultivated *Brassica*. — Jap. Journ. of Genetics V: 151—158.

9. RUDORF, W. 1950. Über die Erzeugung und die Eigenschaften synthetischer Rapsformen. — Zeitschrift für Pflanzenzücht. 29: 35—54.

10. SCHULZ, O. E. 1919. *Cruciferae : Brassica*. — In ENGLER: Das Pflanzenreich IV, 105: 49.

11. SINSKAJA, E. N. 1928. The oleiferous plants and root crops of the family Cruciferae. — Bull. of appl. Bot. and Plant-breed. XIX, 3: 1—648.

12. U, N. 1935. Genome-analysis in *Brassica* with special reference to the experimental formation of *B. napus* and peculiar mode of fertilization. — Jap. Journ. of Bot. 7: 389—452.

13. VILLARS, M. 1786, 1789. Histoire des plantes de Dauphine. I: 350 and III: 334—336. — Paris.

PETER JACOBSEN: Chromosome numbers in the genus *Hedera* L.

The genus *Hedera* has its distribution in the northern temperate and subtropical zones of Asia and Europe. It ranges from the Azores in the west to Japan in the east. In his monograph of the genus *Hedera*, TOBLER (1912) mentions six species and three varieties. The diploid chromosome number for *H. helix* L. f. *typica* is recorded as 44—48 and for the variety *hibernica* KIRCHN. as 88—98 (OEHM, 1924). WANSCHER (1933) gives the diploid number for *H. colchica* C. KOCH f. *arborescens* as approximately 120.

The chromosome numbers recorded in the present paper have been counted on squash preparations of root-tips. Cuttings from the species grown in the Botanical Garden of the University of Copenhagen were made to root in pots in the hothouse. Cuttings of *H. helix typica* have furthermore been taken from a wild population in the Humleore wood, Zealand. It was only possible to procure *H. colchica* as f. *dentata*.

Three technical details should be mentioned by which the chromosomes are sufficiently contracted and spread out to make counting possible: (1) The root-tips are cut off and pre-treated for 6—8 hours with 0,001 mol/l 8-hydroxyquinoline at 12°C, thereby cells with greatly shortened chromosomes in prometaphase appeared in large numbers. (2) Fixation for 18 hours at — 5°C in 70 % alcohol with 1 % lactic acid, which gives very soft cells that are easily flattened out so that the chromosomes are orientated in one plane. (3) The flattening-out of the cells is further improved by using cover glasses and slides treated with silicone.

The diploid chromosome numbers for the species counted are:

Hedera helix L. f. *typica* ..... $2n = 48$
» » » var. *hibernica* KIRCHN. ..... $2n = 96$
» *canariensis* WILLD. ..... $2n = 96$
» *colchica* C. KOCH f. *dentata* ..... $2n = 192$

The chromosomes (8-hydroxyquinoline treated) are biggest in *H. helix* (with the lowest number) and smallest in *H. colchica*. The chromosomes in *H. helix* var. *hibernica* seem to be of the same magnitude as in *H. helix*, and in *H. canariensis* they seem to be medium-sized.

In *H. helix* and *H. helix* var. *hibernica* one pair of satellite chromosomes can be seen, whereas *H. canariensis* has two pairs. Hence it is possible to distinguish *H. helix* var. *hibernica* from *H. canariensis* (which both have $2n=96$) by the number of satellite chromosomes. It should also be noted that the chromosome idiogram of var. *hibernica* with respect to satellite chromosomes does not correspond to that of an (assumed) autotetraploid *H. helix*. In *H. colchica* it was not possible to count the satellites.

H. helix L. f. *typica* ($2n=48$) is distributed over the greater part of Europe and Asia Minor. It ranges between Southern Norway in the north and the Mediterranean in the south, between Ireland in the west and the region around the Black Sea in the east.

In nature *H. helix* L. var. *hibernica* KIRCHN. ($2n=96$) has been found with certainty in Ireland only. Morphologically it is closely related to *H. helix*, and like the latter it has stellate hairs, contrary to *H. canariensis* and *H. colchica*, which species have peltate hairs. From its morphology, distribution, and chromosome numbers it may perhaps be considered a postglacial species originated from *H. helix* autopolyploidy followed by change of chromosome-structure (only one pair of satellites can be seen). In the literature there are no statements about hybridization in nature between these two species.

H. canariensis WILLD. ($2n=96$) is found in nature on the Azores, Madeira, the Canary Islands, and — in North Africa — in Morocco, Algeria, and Tripolitania. Thus it nowhere shares localities with *H. helix* or any other *Hedera* species, as plants of *H. canariensis* north of the Mediterranean and of *H. helix* or *H. poetarum* south of the Mediterranean are all supposed to have been introduced as cultivated plants (TOBLER, 1912). Morphologically *H. canariensis* is more closely related to *H. colchica* than to *H. helix*.

H. colchica C. KOCH ($2n=192$) has its main distribution on the eastern border of the distributional area of *H. helix*, *i. e.* around the Black Sea, the Caspian Sea, Persia, Syria, and perhaps Cyprus. In several places it shares localities with *H. helix*, *H. poetarum* TOBLER, and *H. poetarum*? var. *taurica* TOBLER. *H. poetarum* has its distribution in Southern Greece with an off-shoot to the south coast of the Black Sea. Var. *taurica*, which by TOBLER is considered a probable hybrid between *H. poetarum* and *H. colchica*, occurs in the Crimea. *H. poetarum* has stellate hairs, while the hairs of var. *taurica* show transitions between stellate hairs and peltate hairs. In other morphological characters as well it seems to be of a hybrid nature. There are no statements in the literature about hybridization between *H. helix* and *H. colchica* in localities where these species occur together.

H. himalaica TOBLER, *H. himalaica* var. *sinensis* TOBLER, and *H. japonica* TOBLER have peltate hairs.

On the basis of the four chromosome numbers it is hardly possible to make any definite statement as to the interrelationship of the *Hedera* species. As mentioned above, *H. helix* var. *hibernica* may be a direct descendant of *H. helix*. These two species together with *H. poetarum* constitute a morpholo-

gical group characterized by stellate hairs. *H. canariensis, H. colchica* and all the eastern species constitute another group characterized by peltate hairs. *H. poetarum* ? *taurica* is the only type with hairs of an intermediate structure. Among the *Araliaceae* several species have $2n=24$, hence the hypothesis suggests itself that the *Hedera* species are originated from a *Hedera* (or an *Araliaceae*) with $2n=24$, thus the basic number for the genus would be $x=12$. It seems most likely that from a common diploid ancestor two branches have emerged through polyploid evolution. One branch with stellate hairs comprising the three species, *H. helix, H. helix* var. *hibernica,* and *H. poetarum,* the other one with peltate hairs is represented by *H. canariensis, H. colchica,* and all the eastern species. Chromosome numbers from other *Hedera* species or closely related *Araliaceae* will probably be able to throw light on this question.

Institute of Genetics, University of Copenhagen, Denmark.

Literature cited.

1. OEHM, G. 1924. Studien über Riesen- und Zwergformen einheimischer Pflanzen. I. *Hedera.* — Beih. bot. Centralblatt XL: 257—294.
2. TOBLER, FR. 1912. Die Gattung *Hedera.* — Jena, Gustav Fischer.
3. WANSCHER, J. H. 1933. Studies on the chromosome numbers of the *Umbelliferae.* — Bot. Tidsskrift 42: 384—399.

S. O. BJÖRKMAN: Chromosome studies in *Agrostis.* II.

Some further notes from my cyto-taxonomical investigation of the genus *Agrostis* L. are published here, supplementing the data on the sect. *Trichodium* TRIN. in my previous report (BJÖRKMAN, 1951), and in addition giving information about some species of the sect. *Vilfa* ROEM. et SCHULT.; the sectional names are here used only in a conventional sense.

A. canina L. — Up to now about 600 plants have been investigated with regard to the chromosome number. As far as the Northern, Western, and Central parts of Europe are concerned, the division of the species into the two varieties *fascicularis* ($2n=14$) and *arida* ($2n=28$) is quite correct. — In four different populations of var. *arida* from Northern Sweden pentaploids also occurred.

Material of *A. canina* which differs from the two varieties mentioned above has been obtained from the Iberian Peninsula. One *A. canina* type, morphologically resembling *A. canina* from Northern Spain as reported by PAUNERO (1947), was collected in Northern Portugal (Serra do Gerêz). As regards the shoot morphology, it may be referred to var. *arida,* but its chromosome number is $2n=42$. SOKOLOVSKAYA (1938) states the same number for *A. planifolia* C. KOCH, which has a similar taxonomical position within *A canina* s. lat. as the Iberian type just mentioned.

Other biotypes of *A. canina* from Portugal which can hardly be included in either of the two varieties, had the chromosome number $2n=56$, and regularly showed 28 bivalents. The plants investigated were raised from seeds received from the Botanic Garden, Coimbra, and originating from a natural population.

A. nevadensis BOISS. et REUT. — Twenty-four plants raised from seeds obtained from a natural habitat (Sierra Nevada, Spain) were studied with regard to the somatic number. All counts except two gave $2n=42+ss$; the supernumeraries, occurring in varying numbers (2—10), have a size of about 1/2—1/3 of an A-chromosome. In three cases it was proved that the number of supernumeraries was not constant in one and the same plant. — The two exceptional plants had 43 and 44 A-chromosomes somatically.

A. rupestris ALL. — The material now comprises 34 plants from wild populations in the Alps and 25 from the Pyrenees, and besides these plants raised from seeds obtained from botanic gardens (Copenhagen; München-Nymphenberg) were studied. All specimens from the Alps have the same chromosome number ($2n=28$); supernumeraries do occur, however. The Pyrenean material includes two chromosome races, with $2n=14$ and $2n=28$, respectively. No quadrivalents were observed in P. M. C.'s of the tetraploid. No really good morphological characters distinguishing the two races have as yet been found.

A. Schraderiana BECHERER (syn. *Calamagrostis tenella* LINK.). — Unconditionally an *Agrostis*. Material: 14 plants from the Alps (Switzerland; Alpes Maritimes) and 11 plants from the Pyrenees. $2n=28$, and 14 bivalents in meiosis.

A. borealis HARTM. — The material has been enlarged, and now includes seed plants from Canada (Quebec, Gaspé), all of which showed $2n=56$, the only number found hitherto.

»*A. borealis* HARTM. var. *sabauda* HACKEL». (syn. »*A. rubra* (L.) WG.» sensu auct. gall.). — This type has again been asserted to exist, in notes on finds not only from the Alps but also from the Pyrenees (*cf.* CHOUARD, 1951). — The material examined (the classical specimens from Savoy as well as those recently found) shows clearly that all statements about *A. borealis* (*rubra*) from these places are due to a mistake, since the plant concerned is actually the hybrid *A. rupestris*×*A. Schraderiana*. Specimens collected in the Pyrenees were all growing close to *A. rupestris* populations as well as to *A. Schraderiana*. The cytological examination of »*A. rubra*», giving $2n=28$ and $2n=21$, as well as an analysis of the surrounding *A. rupestris* populations, show that both tetraploid and diploid *A. rupestris* are able to form hybrids with *A. Schraderiana*. The hybrid character of the two »*A. rubra*» types was also demonstrated by the meiosis. — The chromosome number $2n=56$ (CHOUARD, *l. c.*) must be a mistake, unless it was obtained from an amphidiploid between *A. rupestris* (28) and *A. Schraderiana* (28), which may have occurred in the hybrid population.

A. stolonifera L. — The chromosome numbers of about 900 plants were determined. Most were collected in Sweden, but representative collections from Finland, Denmark, Norway, England, Wales, and Scotland were also studied. A few specimens from Switzerland and Iceland were also included in the material. Disregarding the occurrence of supernumerary chromosomes of deviating (smaller) size, the material divides up into three chromosome races: $2n=28$ (about 600 plants), $2n=35$ (160 plants), and $2n=42$ (135 plants). Only two plants were found with aneuploid numbers (33 and 41).

In meiosis the tetraploid forms 14 bivalents; the hexaploid behaves like an

TABLE 1. *Chromosome number in offspring of hexa- and pentaploid Agrostis stolonifera after self-fertilization.*

	Chromosome number																	Number of plants
	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	
No. 1848 ($2n=42$)										1	1	5	1	3*	1	2	—	4	18
No. 1165 ($2n=35$)	1	—	—	1	2	4	3	7	7	3	2	1	1						32

* One plant 40 + 1f; another 39 + 2f.

TABLE 2. *Chromosome number in F_1 of intraspecific crosses in Agrostis stolonifera.*

	Chromosome number (Gametic chromosome number of male side)															Number of plants
	28 (14	29 15	30 16	31 17	32 18	33 19	34 20	35 21	36 22	37 23	38 24	39 25	40 26	41 27	42 28)	
No. 1401 ($2n=28$) × No. 1998 ($2n=42$)	1	—	—	1	3	3	5	6	4	3						26
No. 1090 ($2n=28$) × No. 1007 ($2n=35$)	1	—	—	3	1	1	1									7

autotriploid: at full syndesis forming 14_{III},although first metaphase views with univalents are more frequent. Determinations of the chromosome number of the progeny demonstrate that the hexaploid produced functioning gametes with varying chromosome number (Tables 1—2).

The pentaploid type is also fertile, and a progeny with varying chromosome number was obtained from it: the determinations gave numbers between 28 and 42 (Tables 1—2). A study of the meiosis revealed a varying number of univalents in MI, together with 14 larger associations — bivalents and trivalents. Complete pairing with the configuration $7_{III}+7_{II}$ was also observed.

The hexaploid and the pentaploid were collected in the wild state either separately or together, in localities where the tetraploid occurred but also in localities where the tetraploid seemingly was wanting. No general morphological-taxonomical differences between the three chromosome races have been observed; all three numbers sometimes occur within one and the same morphological type. Most earlier accounts on the chromosome number of true *A. stolonifera* report $2n=28$; SOKOLOVSKAYA (*op. c.*) reports var. *prorepens* with $2n=35$. Reports of $2n=42$ for »*A. alba*» and *A. stolonifera* usually refer to *A. gigantea* ROTH. JUHL (1953) reports, besides $2n=28$, the somatic numbers 30, 32, and 44 for wild plants.

A. tenuis SIBTH. (syn. *A. vulgaris* WITH.). — About 300 plants from natural habitats have been cultivated, and the chromosome number determined. Most (90 %) have $2n=28$, a few were trisomics, and the rest had $2n=28+ss$, the supernumeraries being easily distinguishable by their smaller size. In meiosis there were 14 bivalents; the number $2n=28$ agrees with earlier statements. — STUCKEY and BANFIELD (1946), however, report more or less all numbers between 28 and 42 from plants raised from seeds collected in natu e from plants, which were »presumably Colonial bent». The aneuploid seed plants apparently derive from the natural hybrids *A. gigantea* (»*alba*») × *A tenuis*,

which often occur where the two species meet. The author collected 33 hybrid plants from different localities, all having $2n=35$. The progeny of such a plant exhibits great variation, both morphologically and in the chromosome number (Table 3).

TABLE 3. *Chromosome number in progeny of the back-crosses* [*A. gigantea* × *A. tenuis*] × *A. gigantea* (*I.*) *and* [*A. gigantea* × *A. tenuis*] × *A. tenuis* (*II.*); *the parents bagged together.*

	Chromosome number [The probable chromosome number of the functioning hybrid (female) gamete]															
I.	28 [14	29 15	30 16	31 17	32 18	33 19	34 —	35 14	36 15	37 16	38 17	39 18	40 19	41 20	42 21]	Number of plants
No. 1136 ($2n=35$) × No. 1092 ($2n=42$)								1	10	8	7	8*	3	—	1	38
II.																
No. 1136 ($2n=35$) × No. 956 ($2n=28$)	4	5	5**	3	2	1										20

* One plant 38 + 1f. ** One plant 29 + 1f.

A. gigantea ROTH. — 217 plants from natural habitats were examined, all except two having $2n=42$; the exceptional plants had four supernumeraries besides the normal A-chromosomes. JUHL (*l. c.*) reports, besides $2n=42$, the number $2n=46$. In meiosis *A. gigantea* regularly showed 21 bivalents.

A. castellana BOISS. et REUT. — 9 plants from France (Alpes Maritimes) and 10 plants from Portugal (Serra do Gerêz) were examined. All had $2n=28$; three of the Portuguese plants had 1, 2, and 3 supernumeraries, respectively, the sizes of which were about one third of an A-chromosome. The configuration in MI was 14 bivalents. The number $2n=28$ agrees with that counted by LITARDIÈRE (1950).

A. Reuteri BOISS. — Seeds from the Botanic Garden in Madrid and from Estação Agronómica Nacional, Sacavem (Portugal). $2n=14$.

During my observations of *Agrostis* species, some attention has been paid to the genus *Polypogon* DESF., because of the occurrence of intergeneric hybrids and of species sometimes assigned to *Agrostis*, sometimes to *Polypogon*.

Polypogon semiverticillatus (FORSKÅL) HYL. (syn. *Agrostis verticillata* VILLARS). — Seeds from the Botanic Garden, Lisbon; $2n=28$, which agrees with earlier statements.

Polypogon fugax NEES. — This species often occurs in botanic gardens under several names, especially *P. littoralis* SM., which was originally given to the hybrid *Agrostis stolonifera* L.×*P. monspeliensis* (L.) DESF. — *P. fugax* (seeds from the Botanic Garden in Mainz, Germany, sub nom. *P. littoralis*) had $2n=42$, which agrees with AVDULOV's (1931) and SOKOLOVSKAYA's (*op. c.*) statements on *P. littoralis*. The hybrid »*littoralis*» is stated to have $2n=28$ (RUTLAND, 1941).

The study of the genus *Agrostis* also includes observations on its natural

hybrids and experiments to produce hybrids in cultivation. Up to now the following crosses have been successful (*cf.* JONES, 1953, and DAVIES, 1953). *A. stolonifera* (2*n*=28) × *A. stolonifera* (2*n*=42); *A. stolonifera* (2*n*=28) × *A stolonifera* (2*n*=35); *A. stolonifera* (2*n*=28) × *A. tenuis*; *A. stolonifera* (2*n*=28) × *A. gigantea*; *A. stolonifera* (2*n*=28) × *A. canina* var. *arida*; *A. stolonifera* (2*n*=35) × *A. canina* var. *arida*; *A. tenuis* × *A. stolonifera* (2*n*=28); *A. tenuis* × *A. gigantea*; *A. gigantea* × *A. stolonifera* (2*n*=28); *A. gigantea* × *A. tenuis*; *A. canina* var. *fascicularis* × *A. canina* var. *arida*; *A. canina* var. *fascicularis* × *A. borealis*; *A. canina* var. *arida* × *A. borealis*; *A. borealis* × *A. canina* var. *arida*; *A. rupestris* (2*n*=28) × *A. borealis*; and *A. stolonifera* (2*n*=28) × *Polypogon semiverticillatus*.

Institute of Systematic Botany, University of Uppsala.

Literature cited.

1. AVDULOV, N. P. 1931. Karyo-systematische Untersuchungen der Familie Gramineae. — Bull. Appl. Bot., Genet., Plant-Breed. Suppl. 43. (Zusammenfassung pp. 353—425.)
2. BJÖRKMAN, S. O. 1951. Chromosome studies in *Agrostis*. (A preliminary report.) — Hereditas XXXVII, pp. 465—468.
3. CHOUARD, P. 1951. Nouvelles observations sur l'*Agrostis rubra* (L.) WAHLB. (=*A. borealis* HARTM.) espèce arctique méconnue dans les Alpes et les Pyrénées. — Bull. Soc. Bot. France 97: 10, pp. 119—120.
4. DAVIES, W. E. 1953. The breeding affinities of some British species of *Agrostis*. — Brit. Agricult. Bull. 5, pp. 313—315.
5. JONES, K. 1953. The cytology of some British species of *Agrostis* and their hybrids. — Brit. Agricult. Bull. 5, p. 316.
6. JUHL, H. 1953. An-euploidie und Systematik bei *Agrostis stolonifera* L. und *Festuca rubra* L. aus Schleswig-Holstein. — Ber. Deutsch. Bot. Ges. 65, pp. 331—338.
7. LITARDIÈRE, R. de 1950. Nombres chromosomiques de diverses graminées. — Bol. Soc. Broteriana, sér. 2, 24, pp. 79—87.
8. PAUNERO, E. 1947. Las especies españolas del género *Agrostis*. — Anal. Jard. Bot. Madrid 7, pp. 561—644.
9. RUTLAND, J. P. 1941. The Merton Catalogue. A list of chromosome numbers of British plants. Supplement No. 1. — New Phytologist 40, pp. 210—214.
10. SOKOLOVSKAYA, A. P. 1938. A caryo-geographical study of the genus *Agrostis*. — Cytologia 8, pp. 452—467.
11. STUCKEY, I. H. and BANFIELD, W. G. 1946. The morphological variations and the occurrence of aneuploids in some species of *Agrostis* in Rhode Island. — Amer. Journ. Bot. 33, pp. 185—190.

GÖSTA VON ROSEN: Breaking of chromosomes by the action of elements of the periodical system and by some other principles.

The author has during 1953 continued his investigations (v. ROSEN, 1953) of the ability of different elements to break chromosomes when acting on *Pisum* rootlets.

The method of treatment has been standardized as follows:

Seeds of *Pisum* are rinsed with dist. water and steeped in dist. water in Petri dishes for 24 hours. Then the water is poured off and the seeds are allowed to germinate at 20°C. They are left until the rootlets have reached a length of about 1 cm which takes 1—2 days, depending on the type of seeds used. The compound to be tested is dissolved to a fixed concentration in 100 ml of dist. water. In this solution the seeds are treated for two hours at 20°C, stirring every 30th minute. Then they are quickly rinsed and are given a recovery period of two days between moist filterpapers in a small Petri dish whereafter they are fixed in Carnoy. The Nigrosin method (V. ROSEN, 1949) is used for preparing the slides. Usually 20 rootlets of every treatment are examined under the microscope and normally 300—500 cells are studied in meta- and anaphase stages and chromosome abnormalities are counted.

In all experiments referred to below the chromosome disturbances show the same morphology as those described from X-radiation, isotope radiation or treatment with mustard gas.

The investigations have up to the present given the following information:

(1) The halogenic series of the periodical system induces chromosome breakages. The halogen ion must be electrically attracting. When it is bound in a salt, *e. g.* KCl, Cl' is completely ineffective. Substances such as JCl_3 and S_2Cl_2 are very active. F_2 is most and J_2 least active of the halogenic series. It may be pointed out that the factors for the electrical decomposition tension for the elements decreases in the same order. H_2 is also chromosome breaking.

(2) It is possible to divide the other elements of the periodical system into three groups according to their chromosome breaking ability.

(a) The first group contains Tl, Cd, Cu, Os, Hg, Ag, Ti, Ta, Au, Pt, Cr, and Co, all of which are very active. The activity decreases from left to right. It is interesting to note that these elements include those metals which are known to show a marked chemical complex forming affinity.

(b) The second group contains weakly acting metals, such as Zn, Li, La, Ce, Be, Al, Ni, Fe, Ca, Mn, Sr, Sn. This group is not sharply separated from the former.

(c) To a third more or less inactive group belong, among others, Na, K, Mg, Ba, Pb, Bi and, may be a little surprisingly, also As and Sb. This group of metals contains those which, from a chemical point of view, are very little or not at all complex forming.

The threshold value range at which an active metal gives visible chromosome breakages is normally narrow. The result depends also on the vitality and age of the rootlet and on the age of the seed. The frequency of disturbed cells is between 0 and 20 % with extreme values up to >30 %. The maximum frequency of disturbed cells is normally observed when only a small, or very small, proportion of the divisions have a metaphase-anaphase mechanism that functions, that is, when the greater part of the cells have been "poisoned" in a prolonged prophase. The threshold value range is, for example, for Tl between 10^{-5}—10^{-6} mol, for Cu between 10^{-4}—10^{-5} mol and for Cr between 10^{-3}—10^{-4} mol. After treatment with a stronger solution a more or less total pycnosis in the cell is observed. It is without any significance which negative ion is used in a metal salt, except that a less soluble and less dissociated salt gives

less metal ions and therefore shows a weaker activity than a highly dissociated salt. When, for example, the Cu ion of $Cu(NO_3)_2$ is precipitated with a light surplus of KJ or Na_2CO_3 the test solution totaliy loses its chromosome breaking ability, because very little dissociated Cu salt is now formed. The same result has been observed when this type of experiment has been carried out with Ag, Hg and Li.

From these experiments it is abundantly clear that it is the metal ion itself which breaks the chromosomes. A further investigation has also been carried out to test if the chemical complex forming affinity really is an important common characteristic of the strongly active metals. A great number of metal salts (as nitrate salts) were weighed up in concentrations equivalent to the corresponding concentration of the Tl ion in the Thallium salt. Due consideration was, of course, given to the amount of crystal water in the different compounds. The results provide good evidence that the activity corresponds to the equivalent amount of the metal ion in the solution, *i. e.* the coordination figure of the complex forming affinity, because every optimum in these investigations was found at the same concentration value: 10^{-5} mol (*cf.* Tl above).

When some strongly active metals are added together in the same solution it is necessary to make sure that the total concentration of the ions is kept constant on the threshold value range for the optimum concentration of the metal ions. If the concentration increases, the cells of the rootlets show only pycnosis. When metals are added together, the threshold value ranges remain as narrow as before and the number of disturbances is scarcely increased. Some metals, *e. g.* Ag and Au, produce a great number of swollen prophase cells. In these cells the chromosomes are fully visible as long threads, like *salivary gland chromosomes* of *Drosophila* and they often show great fragmentation. The author is of the opinion that we here have a prophase cell in which the chromosomes are prevented from contraction and, thus, from entering a normal metaphase-anaphase cycle. In this case we should not have a typical *prophase »poisoning«* in the sense of D'AMATO (1952) and others. This effect depends upon a direct action of the complex forming metal ion. The author wants to give it the name *the despiralization type*. It is also typical that it increases in frequency and intensity when more and more metals are added together in the same solution. The maximum manifestation of this abnormality has up to the present been obtained by adding together all metals in a series of eight, namely Tl, Cd, Cu, Hg, Ag, Au, Zn, and Li. On the strength of these observations the author interprets the action of the metal ion as a process of complex formation between the ion and some chemically unsaturated compounds, *e. g.* phosphate- or amino-radicals, which are attracted out into the cell plasm. At the same time the chromosome loses its faculty to contract and to enter a normal division.

It also seems to the author that the results indicate that the number of points in a chromosome in which a metal ion may act, is limited. This activity is not specific for each metal but depends on the coordination figure of the chemical affinity. But every metal also has a specific action on the cell body, the only visible effect of which is a prolonged prophase or *poison effect*. This is to be seen from the very different maxima of the threshold value ranges shown by different metals. Further, there are no results which indicate that two or more metals are antagonistic to each other, *i. e.* show competition about the

same point in a chromosome. It is nevertheless obvious that the despiralization phenomenon is accentuated by the combination of different metals to the same total concentration. This would appear to indicate that a cooperation may take place and the points in a chromosome susceptible to attack may increase when the different activity of the metals are combined. The great sensitivity to a prolonged prophase and the despiralization phenomenon prevent detailed observations.

(3) The chromosome substances are slightly soluble in weak alkaline solutions. At the optimum threshold values chromosome breakages also occur. In solutions with a pH$>$9 the cell enters in pycnosis stage. This phenomenon may sometimes complicate the interpretation of the experiments.

The author would also like here briefly to mention some other causes which during an experiment leads to death or a prolonged prophase stage of the cell and so may obscure the explanations, namely:

(a) Strong concentrations of any salt, normally more than 10^{-2} mol.

(b) The specific action of active metal ions or hydrogen ions of stronger concentrations than the optimum, *e. g.* for Tl$>10^{-5}$ mol, Cu$>10^{-4}$ mol, for the combination of eight active metal ions$>10^{-7}$ mol and for HCl$>10^{-4}$ mol.

(c) A sufficiently strong concentration of some strongly reducing (=despiralizing) metals, as Ag or Au, and from combination of weaker reducing metals.

(d) A weak spontaneous chromosome abnormality.

(e) Varying temperatures during an experiment.

The optimum temperature seems to be 20°C. It has in these experiments also been stated that the vitality of a tissue, estimated as the number of divisions being observed, is strongly correlated with the growth of the root.

(4) Low concentrations of all acids and the corresponding pH-values of acid salts break chromosomes. If the reaction of such active solutions is changed to slightly alkaline, the activity is completely lost. One may thus with a great probability conclude that the hydrogen ion itself is active. But also the oxygen ion from H_2O_2 is no doubt active, and thus one may consider both hydrogen and oxygen as taking part in the oxidation-reduction system in the cell.

From experiments with adding small doses of H_2O_2 to salt solutions of active metals on the one hand and acids, halogenic compounds as JCl_3, compounds which contain SH-groups, *etc.* on the other hand it is clear that there is a fundamental difference in the manner in which these two groups act on the protein. By adding H_2O_2 to the first group the number of disturbed cells is unaltered, but by adding to the second group the average frequency decreases from 11 to 2 %.

(5) When living tissues are placed in a water solution between two platinum electrodes in an electric continuous circuit, but so that the hydrogen- and oxygen-gases which are formed at the electrodes are isolated from the seeds by a water bridge, chromosome breakages are also produced. The frequency of these seems to be proportional to the strength of the current, to the tension, to the specific conductivity of the water solution, and to the time of treatment. That it is not the secondary action of a hypochlorite compound produced by a primary activity of the current in the solution, has been con-

trolled by the author. Although those hypochlorite compounds may be thought able to break chromosomes, some tests with pure hypochlorite compounds did not confirm such an opinion.

(6) When the temperature is changed every day during the growth of the roots chromosome breakages occur. The frequency increases with the temperature differences. It is possible that different varieties of *Pisum* show different sensitivity.

(7) It is well known that several organic compounds induce chromosome disturbances. For comparison some of those compounds have been tested with this method and the result was positive (v. ROSEN, 1953). Special mention should be made of the fact that the SH-group and the alcohol groups have shown activity.

(8) When the roots have to grow *in pure water* as long as the standardized time of treatment lasts or longer, *a low frequency, on an average 3 %*, of *abnormalities* appears. One has to put this phenomenon in connection with the respiration of the roots in water and its influence on the cell enzymes. The disturbed cells show fragmentations and bridges of the usual type. This phenomenon would appear to be comparable to the spontaneous chromosome fragmentation in seedlings of *Vicia faba* which has been described by LEVAN and LOTFY (1950).

The author is of the opinion that the results reported above suggest two different systems of action on the cell substances:

1st: All sorts of active groups mentioned above react with the different proteins in the cell plasm and cell enzymes, thus, directly or indirectly induce chromosome breakages, centromere abnormalities, bridges, *etc.*

2nd: The *complex forming metals* of (2) above and the electrical field of (5) also *react directly with constituents in the chromosome body.* Both may work through *electrical re-loadings* or *"Treffergifte"*. There may be two possibilities for the metal ions to act in the chromosome. Either they form chemical complexes in the chromosome, which would result in a contraction of the body, or they force out of the protein complexes of the chromosome some radical groups and form chemically complex bodies outside in the plasm. This would result in a despiralization of the chromosomes. The observations agree with the last alternative and thus this suggestion seems to be the most plausible one.

Preliminary experiments have already suggested that repeated treatments with varying combinations of different active groups increase the number of disturbances. The result may also be different if they are used together or separated in time. The results indicate that the maximum frequency of disturbed cells is obtained in tissues which have only just survived the treatment. The experiences from experiments with X-radiation, mustard gas, *etc.* have also been confirmed here, namely, that the most sensitive period of the cell cycle is in the early prophase, but it is difficult to catch all cells forming the generative-tissue at the right stage. Taking these facts into account, the author is of the opinion that the chances of obtaining viable chromosome mutations — using the word in its broadest sense — which are capable of being propagated and used in practical plant breeding work, are increased by the combination of widely different methods and active substances which

induce chromosome abnormalities. The treatments must be applied at different times during the period of growth, but first and foremost during the time of meiosis.

The Hilleshög Sugar Beet Breeding Institute, Landskrona, Sweden. December 1953.

Literature cited.

1. D'AMATO, F. 1952. The cytological study of chemical mutagens. — Genetica Iberica IV: 3—20.
2. LEVAN, A. and LOTFY, TH. 1950. Spontaneous chromosome fragmentation in seedlings of *Vicia faba*. — Hereditas XXXVI: 470—482.
3. v. ROSEN, G. 1949. Problems and methods in the production of tetraploids within the genus *Beta*. — Socker 5: 197—217.
4. — 1953. Radiomimetic activity and the periodical system of the elements. — Botaniska Notiser 1953: 140—141.

ARNE HAGBERG: Karyomorphological studies on barley.

In a paper, "Karyomorphology of Cereals", published in Biological Journal of Okayama University, Vol. 1, 1952, TOMOE OINUMA has made a comprehensive and very interesting study of the karyotypes of different cereals. He has especially studied the somatic chromosomes of barley and he has introduced the small letters a—g to designate the seven pairs of chromosomes (pp. 15—16). It is, however, useful and important to have a standard denomination of the barley chromosomes in the ideogramme. TJIO and LEVAN (1950) proposed the figures I—VII: "The pairs I—V are all submedially attached and show a gradual decrease in size from I to V. Some of them, as for instance V, are more asymmetric than others. The two satellited pairs VI and VII, also well-known from other techniques, are easily recognizable." Later in the same year, HAGBERG and TJIO (1950) published an analysis of the karyotype of barley and of a homozygous translocation stock. In this study they used the same numbering of the chromosomes as given by TJIO and LEVAN (*l. c.*). In a paper the following year, TJIO and HAGBERG (1951) gave a careful description of the chromosome pairs of their standard variety "Golden barley" (*l. c.*, pp. 155—157). OINUMA (1952) has cited this paper and a following one (HAGBERG and TJIO, 1952) describing the karyotype of an American barley variety, Mars, and the localization of some translocation points. To avoid mistakes and confusion the present author suggests that TJIO and LEVAN's (*l. c.*) denomination of the chromosome pairs of barley may be accepted as standard. OINUMA's (1952) letters would then be: a=I; b=VII; c=II; d=III; e=IV; f=VI and g=V.

Svalöf in July, 1953.

Literature cited.

1. HAGBERG, A. and TJIO, J. H. 1950. Cytological localization of the translocation point for the barley mutant erectoides 7. — Hereditas XXXVI: 487—491.

2. HAGBERG, A. and TJIO, J. H. 1952. Cytological studies on some homozygous translocations in barley. — An. Aula Dei 2: 215—223.
3. OINUMA, T. 1952. Karyomorphology of cereals. — Biol. Journ. Okayama Univ., Vol. 1: 12—71.
4. TJIO, J. H. and HAGBERG, A. 1951. Cytological studies on some X-ray mutants of barley. — An. Aula Dei 2: 149—167.
5. TJIO, J. H. and LEVAN, A. 1950. The use of oxiquinoline in chromosome analysis. — An. Aula Dei 2: 21—64.